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Overgrowth Syndromes and Disorders: Definition, Classification, and Discussion

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A recent review of the literature made it apparent that a confusing group of disorders with excessive growth and/or development exists. These have been labeled *overgrowth syndromes*. The confusion exists partly because there is no accepted definitive definition or classification of these disorders. The purpose of this article is to present definitions of, and a classification system for, the known types of overgrowth syndromes and disorders.

PROBLEMS WITH DEFINITION AND CLASSIFICATION

Problems with definition and classification of overgrowth conditions are illustrated best by a few examples. One classic overgrowth syndrome is the Beckwith-Wiedemann syndrome. Most children with this condition have excessive prenatal and postnatal growth involving both height and weight, and have macroglossia, visceromegaly, and advanced skeletal maturation. The condition is a classic overgrowth syndrome since there is excessive growth in most growth parameters. Interestingly, however, the head size and presumably the brain size in children with Beckwith-Wiedemann syndrome are normal.

The Marshall-Smith syndrome is another example of the confusion associated with overgrowth conditions. This condition is characterized by a prominent calvarium, forehead, and eyes, a low nasal bridge, an upturned nose, micrognathia, widened middle and distal phalanges, best appreciated on radiographs, markedly advanced skeletal maturation, respiratory distress, frequent pneumonias, failure to thrive, and often death during infancy.¹ Even though the Marshall-Smith syndrome is associated usually

Letter From the Editor

Human growth hormone (hGH) has been used in humans for 35 years (1958-1993). The benefits to growth hormone deficient (GHD) children is well known to all of us. For the first 27 years (1958-1985), we were impressed with the safety of the hormone when it was used in physiologic amounts. In 1985, 2 cases of Creutzfeldt-Jakob disease were described in patients who received hGH many years previously. Subsequently, due to various conjectural reports, concern developed whether hGH produces leukemia, tumor recurrence in the CNS, immunologic deficiency, atherosclerosis, other cardiovascular disease, and/or an increased incidence of diabetes mellitus.

In response to these suspicions, the European Society of Paediatric Endocrinology (ESPE) established a committee of renowned pediatric endocrinologists to review all available data regarding these suspicions and to publish in the official journal of ESPE, *Hormone Research*, a statement concerning the safety and/or toxicity of hGH therapy. This has been accomplished and an official statement from ESPE was published in *Horm Res* 1993;39:92-110. The Lawson Wilkins Pediatric Endocrine Society approved the report.

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with a normal birth weight and failure to thrive, the condition has been classified in the past as an overgrowth syndrome.² This occurred since normally a dramatic increase in the overall bone age is found with the carpal bone maturation being even more advanced.

The Patterson-David syndrome also illustrates the problem in classifying overgrowth conditions. This relatively unknown syndrome has been confused with leprechaunism (Donohue syndrome).^{3,4} Patients with the Patterson-David syndrome present with redundant, loose folds of skin of the hands, large ears, hands, and feet, phallic enlargement in males, generalized bronzed hyperpigmentation, hirsutism, severe mental retardation, and characteristic skeletal changes. In this syndrome the birth weight and length are usually at the 97th percentile; the postnatal length is in the high normal range but the weight typically drops below the 3rd percentile (which probably accounts for the confusion with Donohue's syndrome). Since birth weight and length are increased and since there is redundancy of the skin, large ears, large penis, and large hands and feet, one can make a case that this condition truly is an overgrowth syndrome, despite the postnatal weight deficiency.

A final example includes the localized overgrowth disorders such as isolated macrodactyly. Should these disorders be considered under the umbrella of "overgrowth syndromes"? Perhaps not, but they clearly do represent overgrowth in localized tissues or organs and clearly constitute overgrowth disorders.

DEFINITION AND CLASSIFICATION

Keeping in mind the above examples, I propose that an overgrowth syndrome or disorder simply be defined as a condition in which there is either localized or generalized excessive growth and/or development for the age and sex of the individual. Under this definition, most overgrowth syndromes or disorders can be classified into 1 of the following 3 general categories:

1. Generalized overgrowth syndromes
2. Regional overgrowth disorders
3. Parameter-specific overgrowth disorders

Generalized overgrowth syndromes which include the classic overgrowth conditions are those in which all or most parameters of growth and physical development are in excess of 2 standard deviations (SD) above the mean for the person's age and sex. The relatively few conditions that fall into this category are listed in Table 1. The regional overgrowth disorders include those in which excessive growth is confined to one or a few regions of the body. An example is benign familial macrocephaly, an

autosomal dominant condition associated with a large dolichocephalic head and normal intelligence. Some of the conditions classified as regional overgrowth disorders are listed in Table 2. Finally, there are parameter-specific overgrowth disorders in which a single or, at most, several growth parameters are in excess of normal. Familial idiopathic obesity and Prader-Willi syndrome are examples that belong to this category. Others are listed in Table 3.

Table 1
Generalized Overgrowth Syndromes*

Bannayan-Riley-Ruvalcaba syndrome (Bannayan-Zonana syndrome, Ruvalcaba-Myhre syndrome, or Riley-Smith syndrome) [†]
Beckwith-Wiedemann syndrome [†]
Diabetic embryopathy (infants of diabetic mothers) [†]
Elejalde syndrome [†]
Familial rapid maturation
Familial tall stature
Fragile X syndrome
Gigantism/acromegaly
Hyperthyroidism, congenital
Hyperthyroidism, infancy and childhood
Klinefelter syndrome
Marfan syndrome
Nevo syndrome [†]
Perlman syndrome [†]
Precocious puberty (precocious adolescence)
Precocious gonadotropin-induced adolescence
Congenital adrenal hyperplasia, untreated
Hormone-secreting tumors
Interstitial cell tumor with androgen production in males
Granulosa cell tumor with inappropriate estrogen production in females
Simpson-Golabi-Behmel syndrome (Golabi-Rosen syndrome) [†]
Sotos syndrome (cerebral gigantism) [†]
Teebi-type overgrowth syndrome [†]
Trisomy 8 mosaicism (Warkany syndrome) [†]
Weaver syndrome [†]

* List is not all-inclusive.

[†] Excessive growth and/or weight is usually present at birth in this condition.

The above classification scheme is a modification of the one used by Beighton⁵ who divided overgrowth conditions into generalized overgrowth, obesity, localized overgrowth, and digital overgrowth syndromes. Cohen⁶ also has categorized overgrowth conditions according to whether the condition is a normal variant of growth, such as familial tall stature, or whether the overgrowth is of prenatal onset as in Sotos syndrome, or whether the overgrowth is of postnatal onset as that occurring with early and excessive production of sex hormones.

GENERAL CHARACTERISTICS

Because of the marked diversity of features associated with the various overgrowth syndromes and disorders, no general statements can be made about common characteristics in these conditions. The exception is that they are associated with excessive growth or development of one type or another. However, the conditions in the generalized overgrowth category that have excessive growth at birth (denoted by † in Table 1) share a few common characteristics. These characteristics include the following.⁶

1. Weight is generally increased as much as length.
2. The condition is usually associated with various other anomalies.
3. Mental deficiency often is present.
4. Neoplasias occur at a higher than expected frequency.

INCIDENCE AND NUMBER OF OVERGROWTH SYNDROMES AND DISORDERS

The incidence of each overgrowth syndrome or disorder varies tremendously, being as common as 1 in 1,000 to 1,500 as occurs with the fragile X syndrome, to less than 1 in 1,000,000 births in Elejalde syndrome and others. Elejalde syndrome is a striking prenatal overgrowth syndrome that has been reported in only 3 siblings.⁷

If one accepts the classification system presented above, the number of currently recognized overgrowth conditions is dramatically large. A number of growth parameters and the corresponding number of recognized conditions associated with each of these features is presented in Table 4. These data were generated from 2 syndrome data bases, Pictures of Standard Syndromes and Undiagnosed Malformations or POSSUM and the London Dysmorphology Data Base or LDDb, and clearly indicate the number and complexity of syndromes that can be classified as overgrowth syndromes or disorders.

Table 2
**Conditions Classified as
Regional Overgrowth Disorders***

Cutis marmorata telangiectatica congenita
Familial macrocephaly
Hemifacial microsomia-macrodactyly syndrome
Hemihyperplasia (hemihypertrophy)
Klippel-Trenaunay-Weber syndrome
Macrodactyly
Maffucci syndrome
Neurofibromatosis
Ollier syndrome
Patterson-David syndrome
Proteus syndrome

* List is not all-inclusive.

ETIOLOGY AND PATHOPHYSIOLOGY

A whole gamut of genetic etiologies is associated with overgrowth syndromes and disorders. For instance, an autosomal dominant gene mutation (FBN1) is the cause of Marfan syndrome. The Perlman syndrome is produced by an autosomal recessive mode of inheritance. The Simpson-Golabi-Behmel syndrome is inherited in an X-linked recessive fashion, and familial tall stature is polygenetic in etiology. Genomic imprinting is the usual cause of Prader-Willi syndrome, since

Table 3
**Conditions Classified as
Parameter-Specific Overgrowth Disorders***

Berardinelli lipodystrophy or Seip-Berardinelli syndrome (tall stature and advanced skeletal maturation)
Börjeson-Forssman-Lehmann syndrome (obesity)
Cohen syndrome (obesity)
Congenital contractual arachnodactyly or Beals syndrome (tall stature)
Familial idiopathic obesity
Marshall-Smith syndrome (accelerated skeletal maturation)
Michelin tire baby syndrome (subcutaneous lipomatous nevus)
Prader-Willi syndrome (obesity)
Teebi macrosomia-microphthalmia-cleft palate syndrome (obesity)

* List is not all-inclusive.

the disorder usually is associated with either an interstitial deletion (q11 to q13) of the paternally derived chromosome 15 or maternal uniparental disomy of chromosome 15. In many other overgrowth syndromes and disorders, eg, Proteus syndrome, all reported cases have been sporadic and the etiologies of the conditions are unknown.

Because of the diverse genetic mechanisms causing overgrowth syndromes, and because of the varied manifestations of excessive growth found in individuals with overgrowth conditions, there must be a multitude of mechanisms producing excessive growth. It is indeed intriguing to consider the vast knowledge about human growth and the regulation of cell division and growth that we will have when we come to understand all of the mechanisms producing overgrowth and its associated syndromes and disorders.

Most overgrowth conditions result from either hyperplasia, hypertrophy, an increase in the interstitium, or some combination of these 3 factors.⁶ With the exception of certain hormone disorders such as untreated congenital adrenal hyperplasia, acromegaly, and diabetic embryopathy, the causes for these changes are unknown. Perhaps abnormal states of insulin-like growth factors (IGFs), their cell-surface receptors, insulin-like growth factor-binding proteins, epidermal growth factors, human placental lactogen (chorionic somatomammotropin), and the regulators of these factors cause many of these disorders.^{8,9} In addition, perhaps partial or complete disruption of the normal function of proto-oncogenes or tumor-suppressor genes results in regional overgrowth disorders in some cases, although none is recognized to do so at the present time.

An abnormal accumulation of body fluid, as seen in hydrops fetalis or anasarca, can cause an increase in the size and weight of a fetus or individual. However, these categories of disorders have not been included in the classification scheme presented here since the accumulation of fluid does not truly represent excessive growth in the normal sense of the word.

EVALUATION, COUNSELING, AND FOLLOW-UP

The evaluation of a child with an unrecognized overgrowth condition should be individualized, and based on the type of overgrowth condition present and the presence of other abnormalities. Such an evaluation might normally include: (1) a careful patient history, family history and physical examination; (2) appropriate physical measurements; (3) complete skeletal survey, including bone age; (4) chromosomal analysis that might include specialized testing for specific conditions, eg, fragile X syndrome; (5) urine analysis for metabolic disorders; and (6) endocrine studies, including serum IGF-1 and thyroid function

Table 4
Overgrowth Parameters and the Number
of Syndromes and Disorders Listed in
2 Syndrome Data Bases

FEATURE	Number of Disorders	
	POSSUM*	LDDB†
Macrocephaly	166	137
Macroductyly	8	10
Tall stature	49	44
Asymmetry of the body with hemihypertrophy/ hemiatrophy	36	16
Truncal and generalized obesity	49	80
Excessive birth weight	20	23
Advanced osseous maturation	36	43
Hepatomegaly	91	99
Long and/or large ears	133	77
Large phallus	22	13
Macrotestes	13	8
Large hands	47	23

* Pictures of Standard Syndromes and Undiagnosed Malformations, Version 3.0, 1991

† London Dysmorphology Data Base, 1991

studies. Other studies would be dictated by the patient's history and examination. In many situations it is appropriate to evaluate the parents and siblings of the affected child. It is also necessary to run serial glucose levels on all neonates with generalized overgrowth to detect hypoglycemia. Children with either the Beckwith-Wiedemann syndrome, hemihyperplasia, or the Simpson-Golabi-Behmel syndrome need to be evaluated on a regular basis for intra-abdominal tumors. Finally, appropriate genetic counseling and long-term follow-up care should be provided to both the family and the child with an overgrowth condition.

Extensive summaries of overgrowth conditions, in addition to specific information about these conditions, are found in articles and chapters by Beighton,⁵ Cohen,⁶ and Gorlin and associates.² The reader will find these references of benefit in evaluating the child with overgrowth.

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Letter From the Editor (continued)

The report of ESPE is remarkably thorough and the committee members (E. Martin Ritzén, Paul Czernichow, Michael Preece, Michael Ranke, and Jan Marten Wit) are to be highly commended. Separate consideration in the document is given to each physiologic/pathologic entity under suspicion. Many readers of *GGH* will wish to obtain a copy and review all or part of this report now or at a future date. Its length prevents its publication here; however, the summary that is listed as a foreword in the manuscript, is reprinted here.

Safety of Human Growth Hormone Therapy

Since the introduction of recombinant human growth hormone (GH), this previously scarce drug has become available in technically unlimited amounts. The indications for its use to promote growth has been widened, and many clinical studies are presently under way to test its usefulness also in other conditions than short stature secondary to a proven GH deficiency. Several reports have pointed at possible side effects of GH therapy. Therefore, the ESPE has called on an ad hoc committee to prepare a document describing the present knowledge on proven or suspected adverse effects of GH therapy concerning infectious agents (notably Creutzfeldt-Jakob disease), connection (if any) with malignancies, with disorders of the immune response, carbohydrate and lipid or water/electrolyte metabolism. Mini reviews on these fields are attached to this document as appendices.

Human GH extracted from cadaver pituitaries have been shown sometimes to be contaminated with the infectious agent causing Creutzfeldt-Jakob disease. This has been found to be the case for many different batches of human GH, prepared in many laboratories using different extraction and purification procedures. Due to the very long incubation period of up to 25 years, no single procedure of purification can be proven to be safe until that period has passed. It must therefore be stated clearly that it is no longer acceptable to use any preparation of GH extracted from human pituitaries. On the other hand, there is no reason to believe that recombinant human GH in any way influences the development of Creutzfeldt-Jakob disease or any other infectious disease.

Brain tumors may be the original cause of GH deficiency, and irradiation of the brain as a therapy for tumors may result in GH deficiency. Therefore, many children who have previously had a malignancy are now under treatment with GH. As of today, comparisons of groups of children with such a medical history, with and without subsequent

GH treatment, have failed to show any difference in relapse rate. Leukemia has been reported to be over-represented in Japanese individuals who at some time previously were treated with GH. In worldwide surveys, the slight increase of leukemia observed after GH treatment is only seen when including patients which have other specific risk factors. When such patients are excluded from analyses, children treated with GH do not differ from the general population.

GH decreases the sensitivity to insulin. Therefore, an individual who is in a pre-diabetic state with markedly impaired maximal insulin release might develop clinically obvious insulin deficiency when GH treatment is begun. There is no evidence that GH impairs insulin secretion. Thus, GH may reveal diabetes mellitus but does not cause it.

GH causes immediate and long-term changes in lipid metabolism. Both GH deficiency and excess (as in acromegaly) may predispose to early atherosclerosis. The full effects of normal substitution doses of GH is presently not fully known, but also in this respect it should be of advantage to the growth hormone deficient individual. The consequences of long-term high-dose treatment need further studies.

GH influences the metabolism of water and sodium through the renin-angiotensin system, causing an increase in total body water. This effect is transient and probably dose-related. It is less pronounced in children than adults but may be of significance in patients with impaired lymph drainage, such as Turner's syndrome. If GH is used for patients suffering from heart disease, blood pressure and fluid retention must be monitored during the early phase of treatment.

Most lymphoid cells possess receptors for GH and IGF-I, and GH administration leads to subtle and variable changes in some laboratory parameters of immune function. No clinical symptoms associated with immune dysfunction have been reported in children receiving GH therapy.

In conclusion, human recombinant GH seems to be a remarkably safe drug when used in conventional substitution doses. However, since it is also used in a number of patients with other causes of short stature and at higher than physiological doses, it is important that methods of long-term (decades!) surveillance are developed. In some countries the whole population of GH-treated individuals may be monitored, in other countries representative samples may be followed. Setting up such an organization should be the responsibility of the national health authorities.

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The Etiology and Diagnosis of Overgrowth Syndromes

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Although intrauterine growth retardation is well recognized to be a frequent feature in a number of multiple malformation syndromes, much less attention is paid to overgrowth either as an isolated defect in an otherwise normal individual or as one feature of a multiple malformation syndrome. Since the initial delineation in 1963 of the Beckwith-Wiedemann syndrome (BWS), a number of specific overgrowth syndromes have been described. For most, endocrine and other metabolic studies have not been helpful in explaining the mechanisms of overgrowth. Only recently have some of the newer molecular techniques begun to shed light on etiology. The purpose here is to set forth the principal diagnostic features of the most common overgrowth

syndromes and to present, when available, data regarding etiology and/or developmental pathogenesis. Not covered in this review are 2 rare disorders seen in single families: Elejalde syndrome¹ and Nevo syndrome.² In addition, a number of conditions with asymmetric overgrowth, including neurofibromatosis, Klippel-Trenaunay-Weber syndrome, Proteus syndrome³ and isolated congenital hemihypertrophy,⁴ have not been included.

BECKWITH-WIEDEMANN SYNDROME

Initially delineated independently by Beckwith⁵ and by Wiedemann,⁶ more than 400 cases have been reported. The principal features are set forth in Table 1 and are discussed below.

Growth: Mean birth length for males is greater than the 95th percentile for gestational age. Thereafter length parallels the normal curve at or above the 95th percentile including through adolescence. For females, mean birth length is at the 75th percentile and increases to the 95th percentile by 18 months of age. After 9 years, mean weight remains between the 75th and 95th percentile. Advanced bone age, most pronounced during the first 4 years, only rarely persists until maturity. Spontaneous pubertal development occurs within normal limits for chronologic age and around the 50th percentile for bone age.^{7,8}

Other: Cardiovascular abnormalities, including structural defects and/or cardiomegaly, occur in approximately one-third of patients. Malignant tumors, the majority of which are Wilms' tumor, adrenal carcinoma, and/or hepatoblastoma, occur in about 7% of cases.⁷ An increased risk of malignancy seems to be associated in those children who have hemihypertrophy.³

Etiology: The gene for BWS is located at 11p15.5.⁹ Based on a number of clinical observations, it now seems clear that the characteristic phenotype in this condition occurs as a result of a variety of different genetic mechanisms, all of which result in a dosage imbalance of the gene.^{10,11} Currently, it appears that the maternal copy of the BWS gene normally is imprinted or inactivated. Therefore, there is normally only 1 active copy of the gene functioning at any given time (ie, the paternal copy). Evidence in support of this is set forth schematically in Figure 1 and includes the following: chromosomal abnormalities that cause duplication of the BWS locus at 11p15.5 produce the BWS phenotype when they are paternally derived and, thus, associated with 2 active copies of the gene. Chromosomal inversions and translocations involving the BWS

Table 1
Beckwith-Wiedemann Syndrome

Parameter	Abnormalities
Growth	Prenatal and postnatal overgrowth Accelerated osseous maturation
Craniofacial	Macroglossia Capillary nevus flammeus Prominent eyes with relative infraorbital hypoplasia Prominent occiput Mandibular prognathism Ear lobe creases and/or posterior helical pits
Hyperplasia and Dysplasia	Pancreatic hyperplasia Adrenocortical cytomegaly Large kidneys with renal medullary dysplasia Interstitial cell hypoplasia of gonads Pituitary amphophil hyperplasia
Other	Omphalocele or other umbilical defect GI malrotation Diaphragmatic eventration Cardiovascular defects Cryptorchidism Intra-abdominal tumors Hemihypertrophy Polyhydramnios Neonatal polycythemia Hypoglycemia in early infancy

locus produce the phenotype if they are inherited from the mother. Presumably, disruption of the locus causes activation of a gene that is normally imprinted and thus inactive. Also, the BWS phenotype has been seen in conjunction with paternal disomy, a situation in which both BWS loci are inherited from the father, giving 2 active copies of the gene. Recently Weksberg and colleagues¹² have documented relaxation of imprinting at 11p15.5 in cytogenetically normal, sporadic cases of BWS. These individuals have both maternal and paternal copies of the alleles; however, the maternal copy is inadequately methylated and thus activated.

Another curious finding in BWS that is compatible with the imprinting hypothesis is the observation of an excess of female monozygotic twins discordant for the BWS phenotype. Presumably, this relates to discordant X inactivation in the twins or discordant inactivation at the BWS locus itself. This hypothesis suggests that the process of X inactivation relates to the process of imprinting at autosomal loci. Monozygotic twins with different inactive X chromosomes would also be imprinted differently at the BWS locus, causing discordant phenotypes in a presumed single gene disorder.

A number of endocrine studies have been performed in children with this disorder.¹³ Because of the localization of the insulin-like growth factor 2

(IGF-2) gene to 11p, abnormalities in the insulin-like growth factors would seem to represent the most likely cause of the overgrowth. It is of particular interest that the overgrowth in this disorder is in organs known to be rich in autogenous IGF-2, and that the tumors that occasionally occur in patients with this syndrome demonstrate high levels messenger RNA for IGF-2. Despite this, elevated levels of serum somatomedins have not been found in individuals with this disorder. The hypoglycemia is usually noted in the first 24 hours, but may be delayed to the third day of life, and is due to nesidioblastosis with B-cell hyperplasia and hyperinsulinism.

Although 3 cases of hypothyroidism have been documented, thyroid abnormalities do not explain the overgrowth. Studies of growth hormone, adrenal, and gonadal hormone levels are normal.

SOTOS SYNDROME

The principal features of this disorder¹⁴ are set forth in Table 2.

Growth: Birth length is frequently greater than the 97th percentile while birth weight is usually within the normal range. After rapid linear growth throughout the first year of life, the height stabilizes at or just above the 97th percentile. Although only limited data are available, adult height is usually within the upper normal range.^{15,16}

The difference between bone and chronologic ages gradually increases from 0.6 ± 0.5 years during the first year to 1.8 ± 0.5 years during the fourth and fifth years. Thereafter, the difference stabilizes at 2 to 2.8 years.

A characteristic metacarpophalangeal profile has been established which is based on evaluation of 16 affected patients.¹⁷ Although all had hand bones longer than the mean for normal individuals, the distal phalanges were short relative to the metacarpals and especially to the proximal phalanges.¹⁶

Table 2 Sotos Syndrome	
Parameter	Abnormalities
Growth	Prenatal onset of excessive size Advanced osseous maturation Large hands and feet
Performance	Mental deficiency Poor coordination
Craniofacial	Macrocrania Prominent forehead (dolichocephaly) Frontoparietal balding Down-slanting palpebral fissures Ocular hypertelorism Rosy coloring to cheeks and nasal tip Prognathism with pointed chin High, narrow palate with prominent lateral palatine ridges Premature eruption of teeth
Occasional Abnormalities	EEG abnormalities Kyphoscoliosis Congenital heart defects Neoplasm (5%) Hemihypertrophy Abnormal glucose tolerance test (14%) Thin, brittle nails

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Performance and CNS Defects: Mental deficiency (average IQ, 72) was reported in 85% of individuals affected.¹⁵ Delayed onset of walking and talking almost always is present and clumsiness is common.

Magnetic resonance imaging (MRI) studies of the brain revealed abnormalities of the corpus callosum with complete or partial agenesis or hypoplasia, agenesis of the septum pellucidum and/or septum interpositum, wide or persistent cavum septi pellucidi, hypoplasia of the cerebellar vermi, and large cisterna magna.¹⁸ In addition, in contrast to other disorders associated with macrocrania, individuals with Sotos syndrome have normal sized brains but increased extracerebral and intracerebral fluid spaces.

Etiology: The etiology is unknown. Although the majority of cases occur sporadically in otherwise normal families, at least 5 families have been reported in which both parent and offspring are affected, raising the possibility of autosomal dominant inheritance in certain instances.¹⁹

No consistent endocrine or metabolic abnormalities have been detected, including IGF-1 determinations.¹⁶

WEAVER SYNDROME

Initially described in 1974,²⁰ more than 20 individuals have been reported with this disorder.

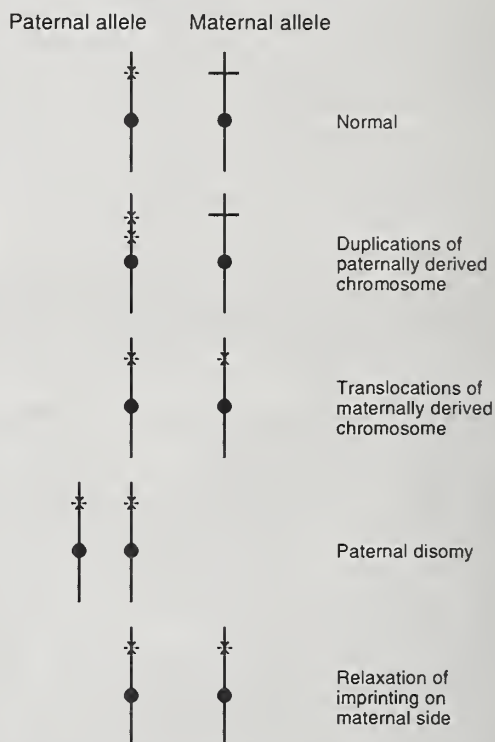
Growth: Although of prenatal onset, overgrowth is not always present by 1 year of age when most reported individuals are at or above the 97th percentile for length and weight. Of the 2 adults reported, 1 male and 1 female, each had a height and weight greater than the 97th percentile. An accelerated bone age is the rule. The carpal bones were more advanced than the phalanges and metacarpals in most cases.^{21,22}

Figure 1



Two-and-one-half-year-old male with Weaver syndrome. Note broad, prominent forehead, down-slanting palpebral fissures, and large ears.

Figure 2
The Etiology and Diagnosis of Overgrowth Syndrome



Performance and CNS Defects: Although a few affected individuals have performed within the normal range, the majority are mentally deficient with IQ scores ranging from 45 to 70. Hypertonia is the rule. One of the affected adults even lost the ability to walk based on progressive spasticity. A hoarse, low-pitched cry is common in infancy. Computed tomography (CT) scans were obtained in 6 cases. Two studies were normal; 2 revealed a cyst in the septum pellucidum. One revealed nonspecific cerebral atrophy and 1 showed enlarged vessels and hypervascularization in the areas of the middle and left posterior cerebral arteries.

Craniofacial Characteristics: Prenatal onset of macrocephaly is common but not invariable, as is the case in the Sotos syndrome.²³ A round face with ocular hypertelorism, down-slanting palpebral fissures, a long philtrum, large ears, and micrognathia are common (Figure 1).

Limbs: Features that distinguish Weaver syndrome from other overgrowth syndromes include camptodactyly, widened distal long bones, and clinodactyly of the toes.²³ Other common features include broad thumbs; thin and deep-set nails; prominent fingertip pads, limited elbow and knee extension, and foot deformities.

Etiology: The etiology is unknown. Although most cases are sporadic, 2 instances of mildly affected mothers giving birth to severely affected sons raise the possibility of either autosomal dominant inheritance with sex-limited expression or X-linked recessive inheritance (Figure 2).

Endocrine studies, performed in at least 12 affected individuals, were normal with the exception of a 6 7/12-year-old boy with hypothyroidism.²⁴

BANNAYAN-RILEY-RUVALCABA SYNDROME

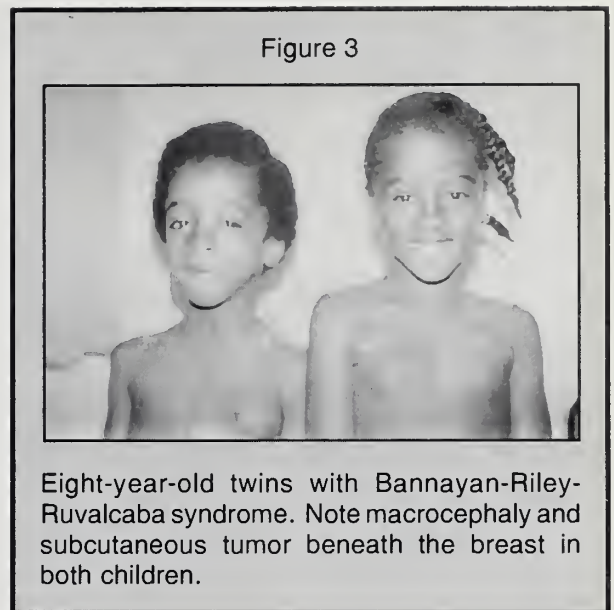
The principal features of this disorder are set forth in Table 3.

Growth: Length and weight which are usually greater than the 97th percentile at birth tend to normalize between 3 and 8 years of age, resulting in normal adult stature. Bone age tends to correlate with chronologic age. Macrocephaly, present in the newborn period, remains as a constant feature in adulthood.²⁵

Performance: Although frank mental deficiency is present in 40-50% of patients, the remainder are of normal intelligence. Electromyographic evidence of a myopathic process has been documented in a number of affected individuals. In some lipid storage myopathy with an increased number of lipid droplets, predominantly in type 1 fibers, was seen.²⁶

Neoplasm: Mesodermal hamartomas, the majority of which are easily resectable subcutaneous lipomas or hemangiomas, occur in approximately 75% of affected individuals (Figure 3). In addition, ileal and colonic hamartomatous polyps have led to intussusception and rectal bleeding.

Etiology: Autosomal dominant inheritance has been documented.



SIMPSON-GOLABI-BEHMEL SYNDROME

The principal features of this X-linked recessively determined disorder include the following:²⁷

Growth: A striking prenatal onset of overgrowth occurs with the birth weight as high as 5.9 kg. In 7 out of 8 affected adults, height was greater than the 97th percentile and ranged from 188 cm to 210 cm. The bone age usually is not advanced. Enlargement of the head, which is present at birth, continues in childhood. Ocular hypertelorism, a short broad nose, a large mouth, and macroglossia are common features. Cleft lip, cleft palate, a midline groove of the lower lip, and preauricular pits and tags are seen less frequently. Unilateral coloboma of the optic disc has been noted in one case.

Performance and CNS Defects: Mental deficiency is variable with the IQ being normal or somewhat delayed. The average IQ is approximately 1 SD below the mean. Hypotonia is common.

Other: Segmental defects of the vertebra are common. Broad halluces and thumbs, postaxial polydactyly of the hands, nail hypoplasia (particularly of the index finger), and partial cutaneous syndactyly of the second and third fingers and toes may occur.

Cryptorchidism and supernumerary nipples occur commonly. Cardiac defects, including bundle-branch block, ventricular septal defects, patent ductus arteriosus, gastrointestinal defects which include intestinal malrotation, pyloric ring and Meckel's diverticulum, and large cystic kidneys occur occasionally.

Etiology: X-linked recessive inheritance has been documented. Recent linkage analysis indicates that the locus for this gene maps to the Xq21.3 region.²⁸ Normal levels of growth hormone and insulin were noted in the 2 affected individuals tested. Partial

Table 3 Bannayan-Riley-Ruvalcaba Syndrome	
Parameter	Abnormalities
Growth	Prenatal onset of excessive size Normal adult size
Performance	Delayed gross motor function Hypotonia Speech delay Mental deficiency
Craniofacial	Macrocephaly with normal ventricular size
Eyes	Prominent Schwalbe lines, prominent corneal nerves, pseudopapilledema
Neoplasm	Mesodermal hamartomas
Genital	Pigmentary skin lesions of glans and shaft of penis

expression, including overgrowth and many of the characteristic craniofacial features, has been seen in some of the obligate female carriers. Approximately one half of the reported patients have died of unknown causes prior to 6 months of age.

DISCUSSION AND CONCLUSION

As more experience has been gained with children affected with various overgrowth disorders, it has become clear that marked clinical similarities exist between them. Many of the diagnostic criteria represent relatively nonspecific growth parameters, and differences in facial features which are often subjective make the differentiation between these syndromes difficult.¹⁸ Therefore, it is important to recognize that certain important generalizations can be made from a practical standpoint when following any child who has overgrowth of prenatal onset. Most importantly, affected children should be evaluated on a frequent basis for the development of tumors. Also important, affected children should be evaluated at an early age since many of these disorders are associated with significant problems that can benefit from early intervention. Finally, it is important to recognize that hemihypertrophy occurs in both BWS and Sotos syndrome. Periodic evaluation for and early recognition of scoliosis secondary to hemihypertrophy hopefully will allow for early intervention and prevention of significant deformity.

In Future Issues

Neuroendocrinology of Growth Hormone Secretion

by Jesus Argente, MD, PhD,
J.A. Chowen, MD

Serum Polypeptide Hormone Binding Proteins

Part 1: Growth Hormone Binding Protein Part 2: Insulin-like Growth Factor Binding Proteins

by Allen W. Root, MD

Insulin-like Growth Factor 2 and Growth

by Yves Le Bouc, MD

Osteochondrodysplasias With Mild Clinical Manifestations: A Clinician's Guide

by Richard M. Pauli, MD

Noonan's Syndrome: A Review

by Michael A. Patton, MA, MSc, MD

Prader-Willi Syndrome: The Unfolding Genetic Story

by Uta Francke, MD

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The Differentiation of Constitutional Growth Delay From Nutritional Dwarfism (ND)

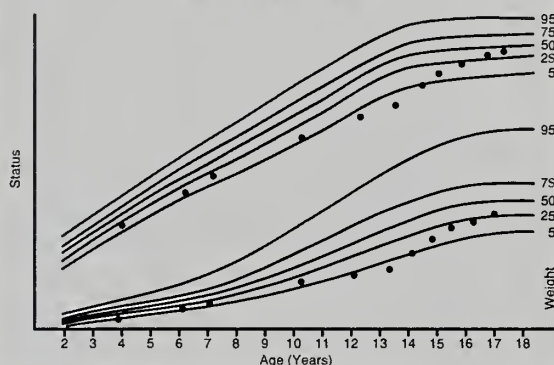
Constitutional growth delay (CGD) is characterized by a retarded linear growth beginning during the first 2 years of life.^{1,2} After age 3 years, the growth of CGD patients typically reveals a consistent progression. There is no further fall-off in height-percentile or impairment of growth over time. A growth spurt occurs during adolescence. There are little data regarding body weight progression in CGD, but weight deterioration has only been reported in the first year of life in these patients.³ On the other hand, in nutritional growth retardation, the longitudinal growth record demonstrates deteriorating weight gain and linear growth.³ Improvement of the deteriorating growth and weight velocity in this type of patient follows nutritional rehabilitation.⁴

The patient's growth chart shown in Figure 1 denotes a child with short stature and delayed puberty who was diagnosed with CGD at age 13 years. Endocrine testing revealed normal GH release to clonidine stimulation and normal thyroid function tests and prepubertal FSH and LH levels. However, *the growth pattern of this patient is not indicative of CGD*. Prior to age 10 years, both height and weight progressed normally. After age 10 years, there was a gradual fall-off in weight and in height. The progressive deterioration of height occurred while body weight gain diminished, although body weight for height remained within the normal range ($\pm 10\%$). The deterioration in weight and height gain coincided with dietary efforts imposed by the mother on herself after she stopped smoking and gained weight excessively. The patient adapted to her mother's diet and eating patterns and failed to gain adequate weight and height or to develop sexually. Nutritional rehabilitation restored weight gain and led to catch-up growth. Therefore, the growth pattern of this patient suggests suboptimal weight gain which is consistent with ND rather than CGD.

We take this opportunity to emphasize that weight and height progression should be evaluated together in any patient with short stature. Nutritional growth retardation should be considered when there is a loss of weight percentile, even when weight for height is normal or when weight is above that expected for height. CGD should not be considered when the patient has a steady decrease in weight gain, except in infancy and early childhood, when these patients manifest deteriorating growth with weight for length deficits.³

Fima Lifshitz, MD

Figure 1
Growth Pattern of Nutritional Dwarfing and Recovery



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Letter From The Editor

The Editorial Board elected to initiate a section in *GROWTH, Genetics, & Hormones (GGH)* called "Clinical Pearls." You each are invited to send us a clinical pearl for possible publication in *GGH*. Please keep it brief, as did Dr. Lifshitz in his writing of the first clinical pearl which is printed on this page. I both hope and believe Dr. Lifshitz' presentation, entitled *The Differentiation of Constitutional Growth Delay from Nutritional Dwarfism* will be helpful in our thinking about growth delay as we see patients in our clinics. You are encouraged not only to contribute pearls yourself *but* to forward comments regarding Dr. Lifshitz' presentation. When sending comments, please write for inclusion of your comments in our "Letter to the Editor" column. We look forward to hearing from you.

Sincerely,
Robert M. Blizzard, MD

Effect of Weight Loss by Obese Children on Long-Term Growth

Epstein et al report on their assessment of height growth in a group of children who were treated in a complex weight loss program 10 years previously. Subjects were enrolled in the original study at 6 to 12 years of age and were initially 20% to 100% overweight for age, sex, and height. The children and at least one parent participated in the weight loss program, which included weekly treatment meetings for 8 to 12 weeks, monthly meetings for 6 to 12 months, and a *traffic light* diet that limited energy intake to 3,780 to 5,040 joules/day. The traffic light diet characterizes foods into color-designated categories, including green foods (primarily low-energy vegetables), yellow foods (basic dietary staples needed for a balanced diet), and red foods (high-energy, low-nutrient-dense foods). Red foods were restricted to 4 servings/wk. When the children were within 10% of ideal body weight, they were placed on a maintenance program and taught to increase their energy intake by 420 J/d per 1 week term until they were no longer losing weight. Subjects were followed prospectively, with anthropometric data collected at 5 and 10 years posttreatment.

One hundred fifty-eight subjects participated in the follow-up studies. Most subjects (80%) were weighed and measured by the principal investigator (PI), while 2% were measured by other physicians since they had moved from the area; 18% self-reported their height and weight. Self-reports were adjusted using regression equations developed by the PI from self-reported estimates of height and weight and measured heights and weights of 1,000 children. Child and midparental height percentiles were constructed based on the National Center for Health Statistics standards.

Mean age at the initiation of weight loss therapy was 10.4 ± 1.6 years; mean height was 71.6 ± 26.5 height percentile; and mean overweight was $45\% \pm 16.6\%$. Initially, boys were more overweight than girls. Height percentiles showed a significant decrease from baseline to 5 years, and 5 to 10 years. Weight also changed significantly over time, with boys showing greater

increases in weight than girls. Height percentiles for boys at 0, 5, and 10 years were 71.3 ± 25.3 , 64.0 ± 27.2 , and 54.6 ± 25.9 , respectively. Height percentiles for boys were significantly greater than midparental height percentiles at baseline and at 5 years but not at 10 years. Height percentiles for girls averaged 71.8 ± 25.2 , 60.5 ± 29.4 , and 58.1 ± 26.2 at baseline, 5, and 10 years, respectively. Height percentiles were also greater than midparental height at baseline and 5 years but not at 10 years. There were no significant differences or changes in height percentiles for successful versus unsuccessful weight maintainers. *Thus, the authors conclude that their weight loss program does not lead to significant long-term reductions in height.* They point out that the accelerated height of obese children is often associated with early puberty and earlier growth spurts, but the final height in these children is similar to their midparental height.

Epstein LH, et al. *Am J Dis Child* 1993;147:1076-1080.

Editor's comment: *This is a very important study. Epstein and colleagues have designed and studied weight loss programs for children in a meticulous fashion for a number of years. Their data have demonstrated some success at weight loss (30% of the children were not obese 10 years after treatment) and now the absence of deleterious effects on final height has been documented. There is concern that children whose energy intake, especially fat, is severely restricted may experience poor growth and delay of puberty. The studies of markedly obese children subjected to moderate calorie restriction and followed prospectively have not been reported previously. The data presented by Epstein et al are reassuring and suggest that greater effort should be made by pediatricians to help children lose excess weight and reduce their risk for obesity-associated disorders of adulthood.*

William L. Clarke, MD

Pharmacologic, Biologic, and Clinical Effects of Recombinant Human Insulin-Like Growth Factor 1 in Growth Hormone Insensitivity Syndromes

The results of coordinated clinical trials of a new recombinant human insulin-like growth factor 1 (rhIGF-1) were presented and discussed in 3 recent papers¹⁻³ issued from a European symposium.

The first paper,¹ resulting from the cooperation of several groups in Europe and the United States, reviews the data acquired on the pharmacokinetics of this new drug in healthy adult volunteers and in young patients with growth hormone receptor deficiency (GHRD) of the Laron type.

In 3 groups of normal males aged 21 to 40 years, the baseline plasma IGF-1 levels varied among individuals from 100 to 200 $\mu\text{g/L}$, with intra-individual day-to-day changes of about 10%; the daily rate of endogenous production, estimated from clearance measurements, varied from 27 to 113 $\mu\text{g/kg/d}$, with a mean of 53 $\mu\text{g/kg/d}$. Following a single dose of rhIGF-1, 20 or 40 $\mu\text{g/kg}$ given subcutaneously after an overnight fast, absorption was slow, with a T_{max} of about 7 hours, and plasma IGF-1 increased in relation to dose, then decreased with a half-

life of about 18 hours. After intravenous injection of 40 $\mu\text{g/kg}$, the increase of plasma IGF-1 was immediate and reached approximately the same C_{max} after subcutaneous injection, and the half-life was near to 22 hours. Daily subcutaneous administration of 20 or 40 $\mu\text{g/kg}$ resulted in a steady trough plasma level slightly higher than the C_{max} reached after a single injection, with a T_{max} significantly shorter and no changes in other pharmacokinetic parameters. No hypoglycemia occurred at any time. Fasting serum insulin levels significantly decreased after injection of the highest dose.

Two male and 4 female adults with GHRD underwent the same pharmacokinetic studies with a treatment of rhIGF-1 40 $\mu\text{g/kg}$ every 12 hours for 7 days. Baseline plasma IGF-1 levels were 16 to 53 $\mu\text{g/L}$. Following a single dose of rhIGF-1 40 $\mu\text{g/kg}$, they reached levels close to 100 $\mu\text{g/L}$, with a short T_{max} of 2 to 4 hours. With twice daily treatment, the mean level of plasma IGF-1 obtained between injections was $141 \pm 34 \mu\text{g/L}$; the half-life was 5 to 7 hours which is considerably shorter than

in normal subjects. The differences between healthy volunteers and GHRD patients are consistent with the low plasma level of the major IGF-1 binding protein, IGFBP-3, in GHRD. These results indicate that if substitution therapy with rhIGF-1 is to be employed in GHRD syndromes, the dosages and dosing rates cannot be based directly on pharmacologic data obtained in normal subjects.

The second paper² reports follow-up studies of IGF-1, IGF-2, and their 3 specific binding proteins during a 6-month study performed in 28 patients with GHRD aged 3.7 to 22.9 years. They were treated twice daily with rhIGF-1 at doses varying from 40 to 120 µg/kg. The acute effects after a first subcutaneous injection of 40 µg/kg confirmed that the pharmacokinetic pattern of exogenous IGF-1 is determined mainly by the IGFBP-3 concentration in plasma. Results after 7 days and 6 months on treatment showed a dose-related increase of plasma IGF-1 measured just before the morning injection, with considerable variations among individual patients. Plasma GH decreased sharply, on the average by 50% after 3 days, with an inverse relationship between GH and IGF-1 in individual samples; levels remained supranormal at 6 months. IGF-2 decreased steadily after 2 months on rhIGF-1. IGFBP-1 showed no significant changes over time. IGFBP-2 increased during the first 2 weeks and remained constant thereafter. Mean IGFBP-3 levels declined slightly but steadily and significantly during the 6 months of treatment.

The whole of results lead the authors to stress the high correlation between IGFs and IGFBP-3 and to speculate that they combine early in the process of their secretion, probably in the liver. Another speculation from the data leads to a tentative

scheme of regulation of plasma IGF-2 and its related binding protein IGFBP-2 by IGF-1 and by the degree of saturation of the IGFBP-3 binding sites by the amounts of IGF-1 available in plasma or liver.

A very short summary of the clinical results obtained with rhIGF-1 in 31 patients is also presented.³ The group of prepubertal children given 10 µg/kg twice daily increased their mean height velocity from 3.9 to 7.0 cm/y during 12 months of treatment. The growth rate of those given 120 µg/kg twice daily only increased from 4.6 to 8.6 cm/y. Main adverse events were injection pain (n=16), headache (n=12), and hypoglycemia during the first 3 months of treatment (n=4).

1. Grahnen A, et al. *Acta Paediatr Scand* 1993;391(suppl):9-13.
2. Blum WF, et al. *Acta Paediatr Scand* 1993;391(suppl):15-19.
3. Wilton P. *Acta Paediatr Scand* 1993;391(suppl):20.

Editor's comment: These 3 reports, each oriented toward different aspects of the same large study of the effects of recombinant IGF-1 in Laron-type GHRD syndromes, are of great physiologic value for the understanding of the growth-regulating hormonal mechanism as well as of clinical interest for the treatment of a rare but severe cause of dwarfism. Each one deserves full consideration as a good example of the power of a well-organized multicenter collaborative study. However, several points of clinical importance are not presented in these papers, and further reports on the experience gained in the long-term management of GH insensitivity syndromes are needed.

Jean-Claude Job, MD

A Constitutively Activating Mutation of the Luteinizing Hormone Receptor in Familial Male Precocious Puberty

Shenker et al hypothesized that familial male precocious puberty (FMPP) might be due to a mutant receptor activated in the presence of little or no agonist (luteinizing hormone [LH]). Genomic DNA was isolated from affected males from 8 different families with FMPP, and polymerase chain reaction (PCR) was used to amplify fragments of the LH receptor DNA encoding amino acid residues 441 to 594. This fragment includes transmembrane helices 3 to 6, the second extracellular loop, and the second and third intracellular loops. Sequencing of the PCR product from one patient showed an adenine (A) to guanine (G) transition at nucleotide 1,733 in codon 578. The mutation (GAT to GGT, T = thymine) encodes a substitution of glycine for aspartate and creates a recognition site for the restriction endonuclease *Msp*I. These results were confirmed by sequencing PCR products from 2 other families.

The functional effect of the mutation was tested with wild-type and mutated human LH receptors (LHR) in COS-7 cells by measuring cyclic adenosine monophosphate (cAMP) accumulation. The mutant LHR was associated with a 4½-fold increase in basal cAMP production indicating that it was constitutively active. The mutant receptor responded to increasing concentrations of human chorionic gonadotropin (HCG) with a 50% effective concentration (EC₅₀) similar to that of the wild-type receptor.

The authors state that constitutive activation of the LHR-mediated cAMP pathway can explain the dominant mutation and pathophysiology of FMPP since testosterone production by Leydig cells is associated with increased production of intracellular cAMP. The authors suggest that the age of pubertal onset in FMPP may depend on the extent to which the mutant allele is expressed as protein, as well as on the relative expression of other genes necessary for Leydig cell maturation. Since both LH and follicle-stimulating hormone (FSH) are required to activate ovarian hormone production, the mutant LHR could not be expected to cause sexual precocity in females.

Six of the 8 families in the study had origins in the same geographic region, and the surname of 1 affected family appeared in 2 of the other family trees.

Shenker A, et al. *Nature* 1993;365:652-654.

Editor's comment: These are exciting findings that not only help explain the pathophysiology of FMPP but also suggest possible mechanisms for other disorders. In the same issue of *Nature* (1993;365:649-651), Parma et al describe similar mutations in the carboxy-terminal portion of the third cytoplasmic loop of the thyrotropin receptor in 3 out of 11 hyperfunctioning thyroid adenomas. These mutations (T to C [cytosine], resulting

in the replacement of Asp 619 by glycine) affect a residue of the thyrotropin receptor. It is homologous to a mutation that leads to the constitutive activation of adenyl cyclase in the α_{10} -adrenergic receptor. In an accompanying editorial entitled "Turned on to Ill Effect" (Nature 1993;365:603-604), Lefkowitz suggests that the mutant receptors as described by Shenker et al and Parma et al displayed properties similar to those of constitutively active mutant adrenal receptors created in vitro. Thus, there are probably many other "turned on" disorders with similar etiologies. Lefkowitz cautions, however, that neither group examined the entire sequence of the receptor for other mutations, and other activating mutations could be present in other regions of the receptors.

This new information could potentially lead to new therapeutic interventions for similar pathologic conditions.

William L. Clarke, MD

2nd Editor's comment: The investigators have convincingly demonstrated that patients with FMPP have an abnormal LH receptor that appears to activate Leydig cell production of testosterone independent of gonadotropin binding. One wonders if the carrier mothers of boys with FMPP are

hyperandrogenic, as only LH is necessary for theca cell synthesis of androgens. Clinically, these women are not hirsute, and they report no difficulty with conception. Although this disorder was associated with the same mutation in all of the patients in this report, there is genetic heterogeneity as different mutations in the LH receptor have been identified in other patients (Laue L, personal communication).

Abnormalities in G-protein activating receptors leading to decreased function have been observed (eg, for the ACTH receptor in familial glucocorticoid insufficiency),^{1,2} this is the first report of a constitutively active receptor. Perhaps other disorders such as Cushing syndrome associated with corticotroph hyperplasia (ie, the CRH receptor) or nodular adrenal hyperplasia (ie, the ACTH receptor) may have a similar pathophysiologic base. In the McCune-Albright syndrome, a mutation in the G-protein itself leads to its independent activation, hyperfunction in multiple tissues, and less selective disease than is observed in a disorder confined to the specific receptor.³

Allen W. Root, MD

1. Tsigos C, et al. *J Clin Invest* 1992;92:2458-2461.
2. Clark AJ, et al. *Lancet* 1993;341:461-462.
3. Shenker A, et al. *J Pediatr* 1993;123:509-518.

Reduction of Bone Density: An Effect of Gonadotropin Releasing Hormone Analogue Treatment in Central Precocious Puberty

Saggese et al determined bone mineral density (BMD) in 13 girls (aged 3.8 to 8.5 years) with central precocious puberty (CPP) before and during a year of therapy with the long-acting gonadotropin releasing hormone (GnRH) agonist D-Trp⁶-GnRH (decapeptyl depot, IPSEN Biotech, Milan). They compared the findings in these girls with data obtained from 2 different control groups: group 1 with 10 prepubertal girls matched to CPP girls according to chronologic age and group 2 with 10 girls matched to CPP girls according to bone age. BMD was determined by single-photon absorptiometry at the distal third of the nondominant radius, and results were expressed as bone mineral content divided by bone width (BMD, g/cm²). Each value was the mean of 3 determinations. Bone age was determined by the method of Gruelich and Pyle.

Prior to treatment, BMD was significantly increased in CPP girls compared with age-matched controls (0.557 ± 0.097 g/cm² vs 0.433 ± 0.09 g/cm²; $P < 0.001$), but was not different from that of bone age-matched controls. Within 6 months of the onset of GnRH agonist analogue therapy, a significant reduction in BMD was observed. A further significant decrease was observed at 12 months. BMD increased as expected during the 12 months in both control groups. The authors state that their findings are similar to those reported for adult premenopausal women treated with GnRH-analogue for endometriosis. They discussed possible reasons why gonadal function may increase bone mineralization, including (1) an action on the parathyroid hormone-vitamin D endocrine system; (2) an enhancement of growth hormone-somatomedin C axis; (3) a direct effect on bone; or (4) a combination of several factors. However, since the CPP girls in the present study continued to grow at an age-appropriate rate (although reduced from pretreatment rates), impaired growth

hormone secretion could not be the sole cause of the findings. The authors plan to continue these studies as they continue to treat their patients and hope to report on whether bone mineralization recovers once therapy is terminated.

Saggese G, et al. *Eur J Pediatr* 1993;152:717-720.

Editor's comment: This paper contributes an important additional piece of information regarding the physiology of bone mineralization during growth and adolescence. It is very important that the authors continue to follow these CPP girls, as well as the control groups, until puberty and final height are achieved in all 3 groups so that the long-term effects of GnRH therapy can be monitored. These findings also suggest the importance of studying BMD in girls with CPP who are receiving recombinant human GH as well as GnRH. Such studies may help identify the relative roles of growth hormone and of sex steroids in bone mineralization.

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Leprechaunism and the Insulin Receptor Gene

Leprechaunism was first described in 1948 by Donohue¹ as a rare autosomal recessive inborn error of metabolism characterized by severe intrauterine and postnatal growth retardation, elfin facies, decreased subcutaneous and muscular tissue, hirsutism, and prominent genitalia.

Patients with leprechaunism have hyperinsulinism due to severe insulin resistance. The insulin resistance in this syndrome has been associated with an inherited defect in a high-affinity insulin receptor.² The central role of insulin is to regulate carbohydrate, lipid, and protein metabolism as well as promote cell growth. Insulin is known to stimulate embryonic reproductive tissue and insulin receptors are expressed very early in embryonic development.

Molecular studies in children with leprechaunism had shown homozygous nonsense or compound heterozygous mutations in the insulin receptor gene,³ but homozygous deletions had never been reported. In all previously described cases, there was some residual function of the insulin receptor, and it was generally believed that complete loss of insulin receptors was incompatible with fetal life.

Recently, Krook et al⁴ and Wertheimer et al⁵ reported DNA

studies on the only patients known thus far to have a homozygous, complete deficiency mutation in the insulin receptor gene. Both patients presented with normal organogenesis, survived beyond term, and had the typical features of leprechaunism.

The authors suggested that although the insulin receptor is important for intrauterine growth, neurologic development and organogenesis can occur in the absence of functional insulin receptors.

1. Donohue WL. *J Pediatr* 1948;32:739.
2. Elsas LJ, et al. *Am J Hum Genet* 1985;37:73-88.
3. Taylor SI, et al. *Endocr Rev* 1992;13:566-595.
4. Krook A, et al. *Lancet* 1993;342:277-278.
5. Wertheimer E, et al. *Nature Genetics* 1993;5:71-73.

Editor's comment: *There may be many complex interactions and buffering mechanisms at work during early embryonic development allowing an embryo with a severe genetic deficiency such as this to survive. Some other as yet unknown pathway must compensate for the absence of insulin receptors.*

Judith G. Hall, MD

Mild to Moderate Zinc Deficiency in Short Children: Effect of Zinc Supplementation on Linear Growth Velocity

In zinc-deficient subjects total body clearance of zinc is increased. Two hundred twenty children with short stature underwent evaluation to rule out evidence of systemic or endocrinologic disorder and to measure zinc clearance kinetics (height for age <-2 SD). Twenty-one prepubertal children had normal serum zinc concentrations but an increased body zinc clearance rate of ≥ 20 mL/kg/h (normal subjects = 15.1 ± 0.6 mL/kg/h). These children were randomly divided into 2 groups, one of which received zinc sulfate 5 mg/kg/d orally for 6 months, the other serving as a control group. After 6 months of therapy, the zinc-treated subjects had significantly increased growth rates (treated: from -3.14 to 2.26 SDS vs controls: from -2.29 to -2.42 SDS) and increased circulating IGF-1 and osteocalcin concentrations in comparison to the control subjects. No cause for zinc deficiency (eg, diabetes mellitus, sickle cell disease, chronic inflammatory bowel disease) was apparent in these 21 children, nor did they have decreased dietary intake of zinc. The authors suggest that measurement of body zinc kinetics may reveal children with mild zinc deficiency. They recommend a trial of zinc therapy in short children with no identifiable abnormality, even if the serum concentration of zinc is normal.

Nakamura T, et al. *J Pediatr* 1993;123:65-69.

Editor's comment: *Zinc deficiency leads to hyposmia and hypogeusia and thus to decreased caloric intake. It has been most apparent in children and adolescents in Middle and Far Eastern countries where zinc intake is low and intestinal absorption is inhibited by zinc binding agents.¹ The present report suggests that mild zinc deficiency occurs in 10 percent of short*

Japanese children. Zinc deficiency in North America has been reported in low income infants and children,² but its frequency in otherwise normal short children may not be high.³ Confirmation of these observations in North American children is necessary before zinc therapy can be recommended routinely.

Allen W. Root, MD

1. Sandstead HH. *Am J Dis Child* 1991;145:853-859.
2. Walravens PA, et al. *Am J Clin Nutr* 1983;38:195-201.
3. Solomons NW, et al. *Pediatr Res* 1976;10:923-927.

2nd Editor's comment: *Zinc deficiency as a possible cause of growth retardation and/or delay in adolescent sexual development first was considered seriously by Prasad working in Iran and, later, Egypt in the 1960s. Subsequently others have considered, and promoted in some instances, zinc deficiency as an etiologic factor in some children with unexplained short stature. I have remained a skeptic because the data have been unconvincing. This study is a credible attempt to clarify the role of zinc in relation to growth. As Dr. Root states, further studies are important and necessary before zinc supplements are used in the U.S. as therapy for growth failure. Six month studies, even when well controlled, are inadequate to derive conclusions that a particular agent is effective in promoting growth. Within the next year, the Editorial Board will invite 2 experts to write point/counterpoint articles regarding zinc deficiency.*

Robert M. Blizzard, MD

Effects of Calcitriol and Phosphorus Therapy on the Growth of Patients With X-Linked Hypophosphatemia

The growth responses of patients with X-linked hypophosphatemia (XLH) to calcitriol and phosphorus (PO_4) therapy in relation to the patients' anthropometric characteristics and/or their pretreatment and posttreatment biochemistries are presented. Twelve consecutive patients whose therapy with calcium (Ca) and PO_4 exceeded 1.2 years were studied. The subjects consisted of 4 females and 8 males, whose ages at initiation of treatment ranged from 1.7 to 9.9 years. Diagnosis in each patient was confirmed by the presence of hypophosphatemia, renal phosphate wasting, normocalcemia, and a normal serum parathyroid hormone (PTH) level. Radiologic evidence of rickets was observed in all subjects except 1 patient, and bone biopsy revealed evidence of osteomalacia in all. Retrospective evaluation showed that 6 patients (group 1) presented with a height below the fifth percentile and 6 patients (group 2) presented with a height exceeding the fifteenth percentile. Sexual development and ages of the children in the 2 groups at initiation of treatment were not statistically different, but ages varied from 4.30 ± 0.98 years in group 1 to 7.00 ± 1.92 years in group 2.

Both groups were treated with calcitriol 30 to 65 mg/kg/d administered in a split dose regimen, and K-phos, 20 to 60 mg/kg/d into 4 divided doses during the waking hours. After adjusting the doses over a period of several months, the optimal combination was administered for a term of 1.2 to 8.1 years. The children were followed at 2- to 4-month intervals. At each visit, 24-h urine and creatinine output were determined and serum Ca and PO_4 levels were measured. Therapy was adjusted to maintain a midmorning serum phosphorus concentration close to 4.0 to 4.5 mg/dL in youths and 4.5 to 5.0 mg/dL in toddlers, while avoiding hypercalcemia and hypercalciuria, which are reflected by a Ca/creatinine (Cr) ratio of >0.25 .

At the initial evaluation, children in group 1 exhibited more severe physical signs; 5 of 6 children had severe bowing of the lower extremities (>5 cm between femoral condyles). This abnormality was present only in 1 of 6 children in group 2 ($P<0.04$). All the children with this finding displayed a marked resolution of the bowing, with reductions of 0 to 2 cm between femoral condyles during the first 2 years of therapy.

Physical manifestations did not correlate with biochemical abnormalities (ie, serum Ca, PO_4 , Cr levels or Cr clearance). However, the younger children in group 1 tended to have a lower serum PO_4 concentration and increased urinary PO_4 excretion.

Both groups required comparable doses of calcitriol (51.9 ± 4.4 ng/kg/d in group 1 and 43.8 ± 6.0 ng/kg/d in group 2). In contrast, those in group 1 required significantly more PO_4 , 47.3 ± 5.1 mg/kg/d vs the group 2 dose of 31.0 ± 4.7 mg/kg/d. In response to therapy, the serum Ca concentration in group 2 increased significantly, but stayed within the normal range. The mean levels were not different from those observed in group 1. The mean urinary Ca excretion in both groups increased during the therapeutic course, but the changes were not significant.

Although serum Cr concentration and Cr clearance in both groups were not significantly different before treatment, urinary Cr clearance declined significantly ($P<0.03$) in children in group 2 and serum Cr increased significantly. Serum PO_4 concentration increased in the majority of the patients. Treatment also enhanced the urinary PO_4 excretion in both groups. The levels

attained during therapy were not different between the 2 groups.

The children in group 1 were shorter than those in group 2. The 6 patients in group 1 presented at a mean height percentile of 1.9 ± 0.6 (z score, -2.2 ± 0.14), whereas those in group 2 presented at a significantly ($P<0.004$) greater mean height percentile of 48.7 ± 8.0 (z score, -0.2 ± 0.8). The short stature in group 1 manifested a decline in the mean height percentile (53.8 ± 12.8 ; z score, 0.12 ± 0.38) in infancy to that present 3.3 ± 1.1 years later at initiation of therapy. In contrast, group 2 children sustained significantly less growth failure. During therapy, patients in group 1 maintained a low mean height percentile of 2.0 ± 0.9 (z score, $-2.3 \pm .24$), which was not different from that before therapy, and exhibited a growth velocity of (z score) -1.05 ± 0.52 . In contrast, children in group 2 significantly ($P<0.03$) increased their mean height percentile to 64.0 ± 9.5 (z score, 0.44 ± 0.25) and exhibited ($P<0.01$) a significantly greater growth velocity (z score, 1.35 ± 0.51).

The authors concluded that the variable growth responses to therapy were not a consequence of the biochemical responses to therapy; that repair of bowing and, presumably, remission of rickets was not related to the variability of growth increment; and that children who were markedly affected with growth retardation at the time of presentation did not significantly increase their growth rate despite improvement in biochemical, radiologic, and other auxologic measures.

Friedman NE, et al. *J Clin Endocrinol Metab* 1993;76(4):839-844.

Editor's comment: This report attempts to elucidate the factors that determine growth response to treatment in XLH, and concludes that clinical and biochemical control are not the main determinants of the growth response achieved with therapy. However, the authors compare XLH patients with different degrees of severity of the disease. Logically, the more severely affected patients will be the most disadvantaged and least responsive to treatment. Although the authors referred to the radiologic evidence of rickets among all patients studied, they failed to quantitate the differences and degrees among the 2 groups described, before and after therapy. It is likely that those patients with more severe disease continued to show bone abnormalities, even after prolonged therapy, which could contribute to the inadequacy of the growth recovery. Similarly, the bone deformities and bowing, and their contribution to the height deficits, were not quantitated before and after therapy. Upper/lower body segments and arm-span assessments, throughout the treatment period, may shed some light on this question. Furthermore, other factors that are important in determining height need to be addressed (eg, height of the patients' parents).

Regardless of the deficits, this paper does point out the need to have a high index of suspicion for XLH so the diagnosis is made early in life and treatment is initiated before bone deformities, rickets, and short stature become evident. Once these signs appear, therapy may not accomplish the desired effects of treatment. An ounce of prevention is worth a pound of cure!

Fima Lifshitz, MD

Effects of Human Growth Hormone Therapy on Melanocytic Naevi

The growth of melanocytic naevi in normal children and in those with hypopituitarism or Turner syndrome, currently or previously treated with hGH therapy, were studied by using HMB-45 antibody which labels actively growing melanocytes. Color slides of the lesions were evaluated using a computerized image analyzer. The growth rate was calculated over 6 months by the change in diameter expressed as a percentage of the initial diameter. In a separate study, 79 naevi were excised from 58 children and adolescents. Of these, 19 of the patients were not presently using hGH and 39 patients were. (The clinical diagnoses were 21 patients with GH deficiency and 18 patients with Turner syndrome.) After fixation of the tissue, studies were done microscopically using HMB-45 antibody which stains the melanocytes.

The mean growth rate of naevi in controls and patients not treated with hGH was 7.6% to 11.2% over 6 months. In patients on treatment with hGH, the growth rate of naevi doubled. Of the 19 untreated or off hGH, 18 patients did not express melanocyte proliferation. Twenty-two of the 39 patients currently being treated with hGH expressed focal or diffuse intradermal HMB-45 antibody reactivity. In one patient with Turner syndrome, the activity correlated with nontreatment and treatment with hGH. The size but not the number of naevi was increased with hGH.

The authors concluded that differences in sexual maturation, age, and diagnosis could not account for the increased growth of naevi. Secondly, the authors felt that the increased HMB-45 antibody expression was not necessarily associated with neoplastic melanocytes and could have resulted from stimulation of normal melanocytes by endocrine or paracrine factors. Thirdly, an increased frequency of skin tumors in hGH-treated or acromegalic patients has not been reported, and no neoplasms or premalignant transformation was observed in the studies reported here. Long-term follow-up is required to identify delayed or unknown effects of hGH therapy, especially in patients with Turner syndrome who are likely to require high doses to obtain substantial growth effect.

Bourguignon JP, et al. *Lancet* 1993;341:1505-1506.

Editor's comment: *The study reported here presents a well-defined effect of hGH on melanocyte activity. The authors are to be congratulated. We all should be more observant of the naevi of patients receiving hGH than has been our practice up to this time.*

Robert M. Blizzard, MD

Growth Hormone (GH) Receptors, GH Binding Protein and GH: An Autoregulatory System?

There is increasing evidence that between the secretion of growth hormone (GH) and its effects on tissues the intermediary steps play an important physiologic role. The view of some authors is that this could constitute an autoregulatory system. Studies in genetically and prenatally GH-deficient dwarfed rats have shown the persistence of sexual dimorphism and with a very low level of GH secretion that is episodic in males and more continuous in females. In these animals the cellular GH receptors (GHRs) exist at a lower level than in normal rats, and develop with age. Continuous infusion of GH increases the GHR binding sites in both males and females, suggesting that GHR autoregulation by GH is preserved in spite of the congenital and permanent lack of GH. Paradoxically, intermittent daily GH injections in the males had a much smaller effect than continuous infusion on plasma GH binding protein (GHBP) and on GHRs. This experimental finding agrees with the observation that normal female rats in whom sustained plasma levels of GH is the norm have higher levels of GHBP than normal males in whom the pituitary secretion of GH is episodic.

Administration of GHBP together with GH, simultaneously or separately, produced a marked prolongation of both GH and GHBP half-lives, compared with their half-lives when injected alone. The experiments with rat and human GH and GHBP showed that the interaction is species-specific. The studies confirmed that GHBP can act as an efficient trap for GH in the circulation, and suggested that complex formation by GHBP in the extracellular space may also serve to trap the extra amounts of GH entering at the time of pulses in the bloodstream, and to prevent this GH from diffusing back into vessels as the plasma concentration of GH wanes.

As continuous GH exposure is not the optimal pattern for growth stimulation in hypopituitary rats of either sex, it may be considered that upregulation of both GHR and GHBP may render the tissues less sensitive to continuous cellular stimulation by GH.

The authors point out that these data obtained in rats do not mean that the same autoregulatory process exists in humans. While the lack of GHRs or the presence of mutated receptors in humans is obviously associated with clinical resistance to GH, it is not clear whether more subtle variations in receptor number or affinity modulate the effects of GH in other conditions. However, the data suggest that continuous treatment, particularly at suboptimal doses, may result in a falloff in GH response, and that intermittent GH therapy might avoid an excessive increase of circulating GHBP.

An example of an increase in GHR and GHBP associated with reduced growth is given with estradiol treatment in normal male rats, which elevates the baseline of plasma GH. This elevation is not sufficient to explain the phenomenon of reduced growth, as continuous GH infusion in hypophysectomized male rats restores the ability of estradiol to raise GHBP and GHR, suggesting an interaction between estradiol and GH in the liver itself to regulate GHBP output. Finally, the authors speculate on the possible clinical significance of their experimental data. They question whether interactions among GH, GHR, and GHBP, and their modulation by sex steroids, could explain certain changes in responsiveness to GH therapy before or during puberty.

Robinson ICAF, et al. *Acta Paediatr Scand* 1993;391(suppl):22-28.

Editor's comment: This interesting report of well-organized animal experiments is potentially important in understanding the effects of chronic GH treatment, particularly the secondary waning of growth velocity, and the changes in dose-response relationships over time or during puberty. However, the role of the autoregulatory system suggested by the authors in rats is

unclear in humans. The messenger role of insulin-like growth factor 1 and its GH-dependent binding protein, possibly the most important step in the whole of the human autoregulatory system, has not been taken into consideration in this experimental work.

Jean-Claude Job, MD

Failure to Improve Height Prediction in Short-Stature Pubertal Adolescents by Inhibiting Puberty with Luteinizing Hormone-Releasing Hormone Analogue

These investigators administered the long acting analogue of GnRH, D-Trp6-GnRH, 3.75 mg intramuscularly monthly for 24 months, to 17 early (breast or genital stage II or III) adolescent subjects (9 females) with intrinsic short stature but no systemic or endocrinologic disorder. The adult height prediction for these patients was below -2.5 SD (females 149.2 ± 3.4 cm; males 160.5 ± 6.1 cm). During administration of D-Trp6-GnRH pubertal progression ceased, testicular and uterine size decreased, and plasma testosterone and estradiol concentrations declined. Unfortunately, pretreatment growth velocity data are not presented, but during GnRH analogue administration growth rates were within the prepubertal range; height standard deviation scores, height age/bone age ratio, and the predicted adult height did not change during therapy. After discontinuation of therapy, pubertal progress resumed in all patients. The major adverse event of treatment was the disappointment of the patients at the lack of effect of the GnRH analogue on their growth potential.

Lindner D, et al. *Eur J Pediatr* 1993;152:393-396.

Editor's comment: Although administration of analogues of GnRH to selected children with central precocious puberty may increase predicted and attained height,¹⁻³ present data indicate that such therapy does not affect the ultimate growth of otherwise normal, short adolescents. Whether co-administration of GH and GnRH analogue would affect adult stature in such subjects is as yet unknown but, even if so, the cost/benefit ratio of such therapy must be carefully considered. In the opinion of this writer, such therapy is not justified in the majority of such subjects until there is unequivocal proof that it is both effective and of psychosocial, educational, and economic benefit. The disappointment of the present subjects over the lack of benefit of treatment is an avoidable complication. Most such patients are best managed by reassurance of their basic normality and individual support.

Allen W. Root, MD

1. Oerter KE, et al. *J Clin Endocrinol Metab* 1991;73:1235-1240.
2. Brauner R, et al. *Eur J Pediatr* 1992;151:728-730.
3. Clemons RD, et al. *Am J Dis Child* 1993;147:653-657.

Evolution of the Sex-Determining Gene

Mammals require a sex-determining gene (SRY) on the Y chromosome for male sex determination and testis differentiation. The SRY gene encodes a protein with a central *high mobility group* domain (HMG box) of about 78 amino acids. In mice, this HMG box consists of an HMG DNA-binding domain flanked by an N- and a C-terminal region.

SRY belongs to a family of genes that are related by sequence homology within the DNA-binding domain. The genes most similar to SRY are called SRY box or SOX genes and are homologous to SRY only in the HMG box sequence. These HMG boxes are found in many proteins, and although SRY is an essential developmental regulator apart from the HMG box domain, the SRY sequence is poorly conserved among species. The conservation of the HMG box in mammals was recently reviewed by Whitfield et al.¹

The authors studied and compared predicted SRY protein sequences in primate (human), lagomorph (rabbit), rodent (mouse), and marsupial (dunnart). Then, by comparing the frequency of DNA point mutations at synonymous and nonsynonymous positions in 8 primates including the human, the authors were able to ascertain the relative frequency of mutations that alter the amino acid sequence and to correct for the evolutionary separation of the compared species.

Whitfield and associates found that there is a high degree of divergence in the flanking sequences of the HMG box, but that the HMG box domain is highly conserved in all species. The results obtained by comparing the differences in the HMG box and the flanking sequences in various species showed that the evolution for the flanking sequences either has been very rapid or may have been the result of directional selection.

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Further studies were conducted by Tucker and Lundrigan² who examined the rate and pattern of evolution of the SRY coding sequence in 7 species of Old World mice and rats. They found varying degrees of differences in the flanking SRY sequences; the HMG box sequences, however, were highly conserved.

These findings suggest that the majority of the noncoding SRY flanking areas (ie, the nonbox regions) may have no functional significance or may have been the result of directional selection. Although unclear, the substitution of amino acids in the flanking sequences must have given the primate SRY gene a selection advantage. It is clear that SRY is constantly being challenged by selective forces and since it plays such a major role in male sex determination, populations with different

SRY sequences may be at risk for reproductive isolation.

1. Whitfield LS, et al. *Nature* 1993;364:713-715.
2. Tucker PK, Lundrigan BL. *Nature* 1993;364:715-717.

Editor's comment: Studying specific genes in evolution allows for further understanding of the critical functional area of each gene. This is especially true in the SRY gene. Clearly the SOX region (the DNA-binding domain) is critical for sex determination, and all evidence so far suggests that sex is sex, regardless of the species.

Judith G. Hall, MD

The Role of Estrogens in Disorders of the Male Reproductive Tract

Diethylstilbestrol (DES) was a widely used synthetic estrogen administered to pregnant women from 1945 through 1971. It was later found to have an in utero teratogenic and a later carcinogenic effect in the daughters of the pregnant women who were exposed to it.

Recent evidence has shown that in utero exposure of males to DES led to an increased incidence of cryptorchidism, hypospadias, falling sperm counts, and testicular cancer.¹ Animal studies also have shown that exposure to synthetic or natural estrogens may lead to male and female reproductive disorders.^{2,3}

Several investigators reported that the incidence of disorders of the human male reproductive tract has increased greatly in the last 40 to 50 years.^{4,5} Sharpe and Skakkebaek⁶ recently wrote a review of the changes that have occurred in the sources of estrogen exposure in humans since 1940. Although an accurate measurement of estrogen exposure levels in humans is not available, this review suggested that the changes that have occurred in estrogen exposure in the past 4 decades may have led to the increase in male reproductive malformations.

Some of the suggestions reported in the review include the possibility that low-fiber diets may lead to the recycling of excreted estrogen, and increased body fat may convert other steroids into excessive amounts of estrogens. Furthermore, the use of synthetic oral estrogens, or the so-called pill for family planning, was not so common 40 years ago. These synthetic estrogens are excreted in the urine and may find their way into drinking water thus increasing human exposure. Orally active estrogens were commonly used in livestock from 1950 to 1970, but were banned in Europe in 1981, and may have been another mechanism for major estrogenic effects.

In addition, increased consumption of dairy products containing estrogens and the increased distribution of environmental estrogenic chemicals⁷ are phenomena that have occurred during the last 50 years and may have altered estrogen exposure of humans.

These changes in the environmental content of estrogens become particularly important for pregnant women. The evidence of male and female reproductive disorders and exposure to DES in utero is very clear.^{1,8} If pregnant women are overexposed to estrogens, then the increase in male reproductive disorders is not surprising.

Because of the association of DES with male reproductive tract abnormalities, and because the routes for human exposure to estrogens have changed so dramatically over time, the

suggestion has been made that the incidence of any disorder associated with estrogen exposure will greatly increase. However, better methods for measuring toxic and nontoxic exposure to estrogens are needed. A good animal model could help solve problems in quantification. Then the association between the increase in male reproductive disorders and the exposure to estrogens could be better understood.

Editor's comment: Sharpe and Skakkebaek's⁶ excellent review of male fetal exposure to estrogens raises several important issues related to side effects of teratogens and the need for in depth evaluation in specific cases. This review also alerts physicians to carefully ask questions about maternal exposure in XY intersex cases and in males with reproductive malformations and malignancies.

Judith G. Hall, MD

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GROWTH

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Neuroendocrinology of Growth Hormone Secretion

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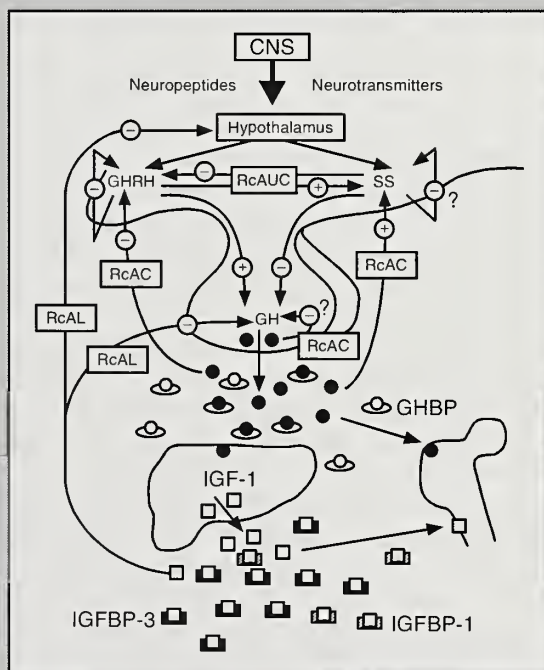
Fundación Endocrinología y Nutrición

Madrid, Spain

The physiology and pathophysiology of growth hormone (GH) was first a matter of discussion in *GROWTH, Genetics, & Hormones* in 1985, with an update of this topic published in 1991. Since this latter article, important advances in our understanding of the GH axis have taken place. Pituitary-specific transcription factors involved in the expression of the GH gene have been identified; the growth hormone-releasing hormone (GHRH) receptor gene has been cloned, as well as a number of somatostatin (SS) receptor genes; pituitary adenylate cyclase-activating polypeptide (PACAP) and its receptors have been described; and advances in our understanding of the insulin-like growth factor binding proteins (IGFBPs), growth hormone-binding proteins (GHBPs), and growth hormone-releasing peptides (GHRPs) have been made (Figure 1). Therefore, the purpose of this article is to review our current

understanding of the control of the GH axis, with a special emphasis on those topics that have come to the forefront since the last review.

Figure 1
Schematic Representation of the Growth Hormone Axis



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Abbreviations

CNS = central nervous system
GHRH = growth hormone-releasing hormone
GHBP = growth hormone-binding proteins
SS = somatostatin
RcAUC = ultrashort loop
RcAC = short loop
RcAL = long loop
IGF-1 = insulin-like growth factor 1
IGFBP = insulin-like growth factor binding protein

NEUROENDOCRINE CONTROL OF GH SYNTHESIS AND RELEASE

It has been more than a decade since the discovery of GHRH and SS, 2 hypothalamic peptides known to play an important role in controlling the synthesis and secretion of GH. Released into the hypophyseal-portal system in a reciprocal manner, GHRH stimulates and SS inhibits GH release from the anterior pituitary. Hence, the pulsatile secretion of GH is thought to result from an increase in GHRH neuronal activity and a coincident decrease in SS release, thus effecting a GH surge, while the opposite (ie, a decrease in GHRH activity and a rise in SS secretion) suppresses the baseline, or nadir. However, how this reciprocal pattern of neuropeptide is generated at the level of the hypothalamus remains an area of intense investigation.

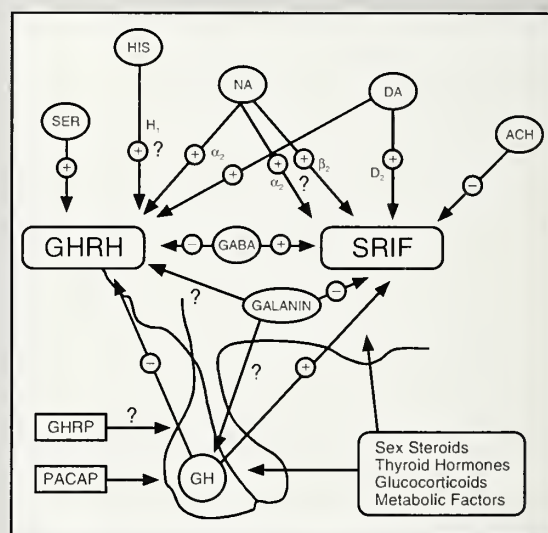
Many neurotransmitters and neuropeptides have been implicated in the control of GHRH and SS release. However, the role of many of these, such as serotonin, γ -amino butyric acid, and dopamine, is still a matter of discussion. For example, dopamine agonists have been shown to both stimulate and inhibit GH release. This depends on the conditions under which these drugs are administered and which agonist is used. One possible explanation for this conundrum is that dopaminergic actions on GH may be mediated through the stress axis or by adrenergic metabolites. The adrenergic system is thought to stimulate GH secretion, acting via α_2 -adrenergic receptors, by increasing the release of GHRH. Clonidine, an α_2 -adrenergic agonist, stimulates GH in humans and in experimental animals, both in vivo and in vitro. However, recent evidence indicates that clonidine may be acting, at least in part, through the inhibition of SS secretion.¹ On the contrary, β -adrenergic agonists inhibit GH secretion, presumably by increasing SS release. The cholinergic system appears to play an important role in modulating GH secretion. Muscarinic cholinergic blockers obliterate both spontaneous and GHRH-stimulated GH release. Cholinergic agonists stimulate GH secretion, most likely by inhibiting SS release. Hence, instead of affecting GHRH secretion, many of the higher pathways appear to modulate basal hypothalamic SS release.

Over the years, a number of neuropeptides have been implicated in the control of GH secretion, both at the level of the hypothalamus and the anterior pituitary (Figure 2). Thyroid-releasing hormone, vasoactive intestinal peptide (VIP), gastrin, neurotensin, and substance P, among others, have been suggested as stimulators of GH secretion, while calcitonin, neuropeptide Y, and corticotropin-releasing hormone (CRH) have been implicated in the diminution of GH secretion. Galanin, a 29 amino

acid peptide found in considerable amounts in the hypothalamus, both stimulates GH release and enhances the GH response to GHRH release. This peptide is thought to act by diminishing the naturally occurring SS inhibitory tone. Interestingly, galanin is also produced by a percentage of GHRH neurons and can be found in the median eminence, suggesting that this peptide may have a biologic function in the control of GH secretion.

As a newly isolated hypothalamic peptide with a possible role in the control of GH secretion, PACAP has received considerable attention.² This polypeptide is a member of the secretin-glucagon-VIP family, with the greatest homology to VIP. It is present in 2 amidated forms, one with 38 residues

Figure 2
Effects of Hypothalamic Neurotransmitters on the Release of GHRH and SRIF and on the Secretion of GH



Metabolic factors (hypoglycemia or hyperglycemia, fasting) as well as sex, thyroid, and glucocorticoid hormones act in both the hypothalamus and anterior pituitary to modify the secretion of GH.

Abbreviations

SER	= serotonin
HIS	= histamine
NA	= noradrenalin (norepinephrine)
DA	= dopamine
ACH	= acetylcholine
GABA	= γ -amino butyric acid
GHRH	= growth hormone-releasing hormone
GHRP	= growth hormone-releasing peptide
SRIF	= somatotropin release-inhibiting factor (somatostatin)
PACAP	= pituitary adenylate cyclase-activating peptide
GH	= growth hormone

(PACAP38) and one with 27 residues (PACAP27); PACAP38 is the more abundant form. As its name suggests, this polypeptide acts to stimulate cyclic adenosine monophosphate (cAMP) activity, but not only in the pituitary. A number of different tissues, including the hypothalamus, express receptors for PACAP. To date, 2 types of PACAP receptors have been identified. Type I has 2 subforms. Type A binds to both PACAP38 and PACAP27, with a slight preference for the latter; type IB has a greater preference for PACAP38. The type II receptor binds both PACAP and VIP with similar affinities, and may be identical to the VIP receptor. This receptor is found in lung, liver, intestine, and other tissues; the type I PACAP receptor is found in high concentrations in brain, spinal cord, anterior pituitary, and adrenal medulla. Although the precise role that this peptide plays in the regulation of GH secretion remains to be elucidated, evidence is accumulating to suggest that it may indeed be another hypothalamic factor involved in this phenomenon. Not only does the anterior pituitary contain type I PACAP receptors, but immunoreactivity for PACAP can be found both in the hypothalamus and median eminence. Furthermore, studies have demonstrated that this peptide can increase the calcium concentration in somatotropes and stimulate the release of GH from anterior pituitary cell cultures.³ However, a possible role for PACAP in human short stature remains to be elucidated.⁴

Synthetic hexapeptides that stimulate GH release, GH-releasing peptides 1, 2, and 6 (GHRP-1, GHRP-2, and GHRP-6), have been available for a number of years.^{5,6} These peptides are not homologous to GHRH and now are known to work through anterior pituitary receptors distinct from those of both GHRH and PACAP. In normal children, these peptides stimulate the release of GH, acting directly at the pituitary level. Although the mechanism is not yet understood, GHRPs do not activate GHRH receptors.⁷ In addition, evidence has accumulated in the literature indicating that GHRPs and GHRH act in a synergistic manner when administered simultaneously. Administered orally, GHRP-2 can be used as a potent drug to liberate GH from somatotropes. Recently, a nonpeptidyl substituted benzolactam has been developed that acts in a manner similar to that of GHRP-6 and is biologically active in humans. A possible role for GHRPs in the treatment of human short stature remains to be demonstrated.

Many circulating hormones and metabolic substances affect GH secretion, a number of which act, at least in part, at the level of the hypothalamus. Thyroid hormone, acting at the level of both the pituitary and the hypothalamus, plays an important role in the regulation of GH production and secretion. The promoter of the rat GH gene contains a

thyroid hormone-response element (TRE) and is induced by thyroid hormone. However, this triiodothyronine response element has not been identified in the human GH promoter; moreover, human GH gene transcription appears to be negatively regulated by triiodothyronine (T_3). In humans, both hypothyroidism and hyperthyroidism can lead to decreased GH secretion. This may be the result of a dual action of thyroid hormones, decreasing hypothalamic somatostatinergic tone and antagonizing GHRH action at the level of the pituitary.

In humans, glucocorticoids are important regulators of GH secretion. Administration of glucocorticoids to both humans and laboratory animals has been shown to blunt GH secretion and to inhibit somatic growth. This observation is counterintuitive when one considers more recent evidence showing that glucocorticoids stimulate the GH gene at a specific corticoid-response element. Indeed, glucocorticoids also appear to have dual effects on the GH system. Although a single dose of dexamethasone is capable of blocking GHRH-stimulated GH secretion for several days, glucocorticoids potentiate GH secretion over the short term (3 hours). It may therefore be that the stimulatory and inhibitory effects of glucocorticoids are mediated at different levels of the GH axis.

It has long been known that there is an interrelationship between sex steroids and GH. The spontaneous elevation of sex steroids during puberty or the administration of small or moderate doses of androgens or estrogens during the prepubertal period results in a marked increase in the response of GH to pharmacologic stimuli, in addition to augmenting the spontaneous basal secretion of this hormone. At least in the laboratory rat, androgens stimulate the synthesis of both hypothalamic GHRH and SS,⁸⁻¹⁰ suggesting that the effects of sex steroids are at least partly mediated at the hypothalamic level. Gonadal steroid effects at the pituitary level are less clear, although they may exist.

GH secretion is profoundly modified in metabolic diseases such as anorexia nervosa, obesity, malnutrition, and diabetes mellitus. This is not surprising considering the number of metabolic substances that modulate GH secretion. One commonly used diagnostic test for GH secretion abnormalities involves inducing hypoglycemia. Hypoglycemia itself normally produces a rise in GH secretion, while insulin inhibits GH release, an action that may be mediated at the hypothalamic level since insulin receptors can be found in this tissue. Furthermore, free fatty acids inhibit and amino acids stimulate GH release. Delineating the precise mechanisms underlying abnormal GH secretion in these metabolic diseases remains an area of active research.

PITUITARY TRANSCRIPTION FACTORS

The identification of the pituitary-specific transcription factor, Pit-1 (or GHF-1), in 1988 has contributed substantially to our understanding of the physiology and pathophysiology of GH synthesis.^{11,12} This transcription factor is involved in the developmental generation of somatotropes, lactotropes, and thyrotropes, as well as in the regulation of the genes for GH, prolactin, and possibly the β -chain of thyroid-stimulating hormone.^{13,14} Some effects of GHRH and SS, both of which act by modulating cAMP levels in the somatotrope, may be mediated through Pit-1. Although no cAMP-response element (CRE) has been demonstrated in the GH gene, the promoter region of the Pit-1 gene contains a CRE, and the transcription of this gene is augmented with increasing levels of cAMP. Furthermore, the interaction of this protein with other DNA-binding factors, such as sex steroid receptors, thyroid hormone receptors, and glucocorticoid receptors, may also modulate the response of somatotropes to these other circulating factors. Alternative splicing of the messenger RNA for Pit-1 results in a related transcription factor, Pit-2.¹⁵ This peptide is also endogenous to the anterior pituitary, although it is found in a concentration 7-fold less than that of Pit-1. The physiologic role of Pit-2 is even less well understood than that of Pit-1, although it appears to be much more potent in stimulating the transcription of the GH gene than of the prolactin gene.

The cloning of the human cDNA for this transcription factor has led to the clinical classification of a new type of pituitary insufficiency. During the past year, a small number of patients has been described in whom the underlying deficit includes different mutations involving most of the exons in the gene for Pit-1.¹⁶⁻¹⁹ These patients present with severe short stature, an absence of GH and prolactin production, and different degrees of thyroid deficiencies and pituitary hypoplasia. The demonstration of this new combined pituitary hormone deficiency syndrome will allow pediatric endocrinologists to identify the underlying deficit in these patients. An excellent review has been recently published by Parks and colleagues.²⁰

Because the GHRH receptor gene has been cloned,²¹ molecular diagnosis of short stature, including GHRH receptor abnormalities, can be made. Although, this defect has not yet been demonstrated, possible candidates were recently presented at the Fourth Joint Meeting of the Lawson Wilkins Pediatric Endocrine Society/European Society of Pediatric Endocrinologists (LWPES/ESPE) in San Francisco. These extremely short-statured children do not respond to exogenous GHRH and exhibit very low levels of IGFBPs. Mutations in or near the GH-1 gene were excluded.²²

IGFBPs AND GHBPs

The insulin-like growth factors (IGFs) circulate in serum bound to IGFBPs. During the last few years new biochemical parameters, including the IGFBPs and the GHBPs, have become available to the clinician in order to better evaluate normal growth and growth disorders during childhood.

The IGFBPs are circulating proteins that modulate IGF actions.^{23,24} Based upon protein and DNA sequence analysis, 6 different human IGFBPs have been classified so far: IGFBP-1 through IGFBP-6.²⁵ The major circulating IGFBP can be detected in serum as a 150-kilodalton (kd) ternary complex. The IGFBP subunit (β) of this complex is IGFBP-3, a 45-kd glycosylated protein with a core molecular mass of 29 kd. The α -subunit is a glycosylated protein of 85 kd that is unable to bind IGFs. The third component, or γ -subunit, is IGF-1 or IGF-2. Changes in IGFBP-3 levels during childhood have been described. IGFBP-1 is generating much interest because it possesses properties that are atypical for a classic binding protein.²⁶ Its physiologic role remains unknown, although IGFBP-1 could be the endocrine element secreted by the liver under insulin regulation in order to modulate IGF activity in humans. IGFBP-1 levels vary markedly during the day.²⁷ This variation is related to metabolic status, with an inverse relationship existing between IGFBP-1 and insulin levels. In contrast, little is known about IGFBP-2, IGFBP-4, IGFBP-5, and IGFBP-6.

The identification and characterization of the GHBPs, as well as the cloning of the GH receptor,²⁸ have provided new biochemical markers useful in understanding the physiology of GH and growth disorders.²⁹⁻³¹ Serum GHBP levels correlate inversely with 24-hour GH secretion in healthy boys of normal stature.³² In addition, there is a strong positive correlation between the body mass index (BMI) and serum levels of high-affinity GHBP in normal boys. Furthermore, there is a significant direct relationship between BMI and responsivity to exogenous GH,³³ suggesting a relationship between GH function and circulating GHBP levels.

SUMMARY

The rapid advances that have occurred in biochemistry and molecular biology have led to an enormously increased understanding of the GH axis. However, this increase in knowledge has led to even more questions and has complicated our theories regarding the control of GH secretion. Nonetheless, these new diagnostic tools have helped to define new diseases, such as the combined pituitary hormone deficiency syndrome, and to improve our understanding of the variety of diseases involving growth abnormalities. As new genes

involved in the control of the GH axis are cloned, the possibilities for future diagnosis and identification of underlying defects will improve. Our increasing understanding of peptides such as PACAP and the GHRPs will likely play an important role in the years to come regarding the diagnosis and treatment of GH abnormalities.

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Serum Polypeptide Hormone-Binding Proteins Part 1: Growth Hormone-Binding Proteins

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Serum proteins that bind polypeptide hormones have only recently been identified. The recognition of these serum proteins has necessitated revision of concepts concerning the mechanism of action of their ligands, as these binding proteins alter the metabolism and influence the association of the polypeptide hormones with their cell membrane receptors. They also may enhance or inhibit the bioactivity of their ligands. The most well-characterized binding proteins are those for growth hormone (GHBP) and the insulin-like growth factors (IGFBPs).

GROWTH HORMONE-BINDING PROTEINS

Serum proteins with GH-binding activity have been identified in humans, rabbits, mice, rats, swine, and higher primates.^{1,2} Two GHBPs are found in human serum, one with low affinity and the other with high affinity for GH. The low-affinity, high-capacity GHBP with a molecular weight (MW) of 165 kilodaltons (kd) specifically binds the 20-kd variant of GH. The high-affinity human GHBP is discussed here unless otherwise indicated. This protein binds primarily the more abundant 22-kd form of GH. Structurally it is

identical to the extracellular domain of the membrane receptor for GH.

In rodents, GHBP is synthesized from an alternatively spliced mRNA transcribed from the gene for the GH receptor; it contains an extra hydrophilic sequence that replaces the transmembrane and intracellular domains of the GH receptor. However, in rabbits, and probably in humans, GHBP is synthesized by proteolytic cleavage of a single transcriptional/translational product of the GH receptor gene³ and is a 239 to 246 amino acid glycoprotein (MW 61 kd) primarily of hepatic origin.

Serum or plasma GHBP values have been estimated by determining the percentage of protein-bound radiolabeled GH after incubation of radiolabeled GH with serum and separation of protein-bound from free GH by exclusion or high performance liquid chromatography. Methods for separation of protein-bound and free GH by adsorption of free GH to charcoal or immunoprecipitation of protein-bound GH with monoclonal antibody to GHBP or to the GH receptor as well as a radioimmunoassay for (rodent) GHBP have also been described.^{4,5} Both total GHBP (free and GH-bound) and the endogenous complex of GH and GHBP (GH/GHBP complex) may be measured by utilizing the ligand-mediated immunofunctional assay (LIFA).^{6,7} There is a reasonable correlation between LIFAs and radiolabeled GH-binding assays, although absolute GHBP measurements by LIFA are one third of the values obtained by the radiolabeled GH binding assay.⁸

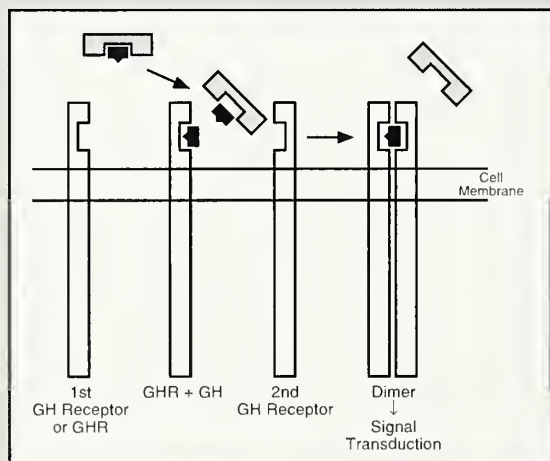
In both males and females, approximately 40% to 50% of circulating GH is bound to high-affinity GHBP and 5% to 8% to low-affinity GHBP, leaving 42% to 55% free. Total GHBP is relatively constant throughout a 24-hour period, although slightly lower at night than during the day. Levels of GH/GHBP complex fluctuate throughout the day, paralleling the changes in endogenous GH secretion.⁷ In the fetus and neonate, GHBP values are low, increase 2-fold by 6 years of age, and continue to rise throughout childhood. During adolescence, GHBP values do not change, although values may fluctuate 3-fold over time in an individual.⁹ In children and adolescents the level of GHBP is directly related to height, weight, and therefore, body mass index. Serum levels of GHBP are low in malnourished subjects, high in obese individuals, and decline with weight reduction.⁵ GHBP levels are similar in normal, GH-deficient, and acromegalic adults. Administration of GH does not alter GHBP values in normal or GH-deficient adults,^{2,10} suggesting that GH does not influence the production of GHBP. However, in GH-deficient children an increase in GHBP values after administration of GH has been reported. Whether this response is due to the effect of GH itself or to the increase in body size is unclear.^{11,12} Testosterone decreases and estradiol increases GHBP levels, which is consistent with its hepatic origin.¹⁰

Although GH probably does not directly regulate the production of GHBP, GHBP does appear to influence the secretion and biologic activity of GH. Thus, there is an inverse relationship between the GHBP level and the mean 24-hour GH concentration, mean amplitude of the GH pulse, and the sum of the amplitudes of the GH pulse in normal children and adolescents. In GH-deficient children, the linear growth response to a constant dose of GH is inversely related to the pretreatment GHBP value.^{9,13,14}

GHBP serves as a reservoir for secreted GH; the half-life of free GH in serum is approximately 7 minutes, and that of bound GH/GHBP complex is 27 minutes. The half-life of total GH is 18 minutes. In this dynamic system, the minute-to-minute concentrations of free GH, GHBP-bound GH, total GH, and the percentages of free GH, GHBP-bound GH, and occupancy of GHBP vary with the half-life of free GH, the instantaneous secretion rate of GH, and the capacity and affinity of GHBP.¹⁵ Although the GH receptor is a single-chain polypeptide, effective induction of the intracellular GH signal requires that 2 separate sites of the circulating GH molecule interact with 1 specific receptor binding site, which is near the amino terminal of the extracellular domains on 2 GH receptor molecules.^{16,17} GHBP may facilitate the exposure of (GHBP-bound) GH to the extracellular domain of the cellular GH receptor (Figure 1). In vitro, GHBP inhibits the binding of GH to its receptor and attenuates the biologic effectiveness of GH.¹⁸ In vivo, GHBP appears to enhance the growth-promoting effects of GH in humans and rats.^{14,19} Clark et al¹⁹ demonstrated that recombinant human GHBP had no effect on the growth of GH-deficient dwarf mice, but more than tripled the effect of a constant dose of human GH on bone growth and weight gain, an effect attributed to prolongation of the effective biologic half-life of GH. These observations are difficult to integrate into the concept that pulsatile secretion of GH is important for optimal GH effect. Thus, excellent growth and a final height consistent with the genetic background can be achieved by administration of GH once daily to GH-deficient subjects. Interestingly, continuous infusion of GH stimulates linear growth to the same extent as intermittent injection,¹² and overgrowth occurs in patients with GH-secreting tumors in whom GH levels may be relatively constant. The exposure of tissues to coordinated levels of constant basal and intermittently increased amounts of free GH could conceivably be optimal for cell growth and function.

In many subjects with GH insensitivity due to an abnormality in the gene for the GH receptor, such as in the Laron syndrome, serum GHBP activity is low

Figure 1



Growth hormone-binding protein (light gray symbol) delivers growth hormone (GH, solid symbol) to a single binding site on the extracellular domain of 1 cell membrane GH receptor (GHR). Two molecules of GHR then dimerize. The univalent binding site on the extracellular domain of the 2 receptors binds to 2 different portions of the GH molecule. Dimerization is essential for signal transduction of the GHR.^{16,17}

or absent. GHBP values may also be low in patients with cirrhosis of the liver, chronic renal disease, insulin-dependent diabetes mellitus, malnutrition, and severe acute illness.^{2,20-22}

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Serum Polypeptide Hormone-Binding Proteins Part 2: Insulin-Like Growth Factor-Binding Proteins

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The family of serum proteins that specifically bind 7.5-kilodalton (kd) insulin-like growth factor (IGF) 1 and 2 (but not insulin) whose gene structures and amino acid sequences have been identified currently numbers 6 members (Table 1).¹⁻³ The amino terminal, cysteine-rich 80-90 and the carboxyl terminal 80-100 amino acids of the IGF-binding

Table 1
The Insulin-Like Growth Factor-Binding Proteins

IGFBP	Chromosome	Mature Protein	Source	IGF-Binding Characteristics
IGFBP-1	7p12-14	234* 25 kd	Amniotic fluid Human serum Placenta Endometrium	IGF-1=IGF-2
IGFBP-2	2q33-34	289 31 kd	Human/rat serum Fetal serum Cerebrospinal fluid	IGF-2>IGF-1 (10:1)
IGFBP-3	7p12-14	264 29 kd	Human/rat serum Liver Fibroblasts Follicular fluid	IGF-1=IGF-2
IGFBP-4	17q12-21	237 26 kd	Osteosarcoma Prostatic carcinoma Human/rat serum	IGF-1=IGF-2
IGFBP-5	5	252 29 kd	Osteosarcoma Human bone Cerebrospinal fluid Human/rat serum	IGF-2>IGF-1 (5-15:1)
IGFBP-6	12	216 23 kd	Fibroblasts Cerebrospinal fluid Human/rat serum	IGF-2>IGF-1 (70:1)

* Number of amino acid residues
Adapted from references 1-3.

proteins (IGFBPs) have substantial (50% to 80%) sequence homology within and between species. IGF-binding regions of IGFBP are located near cysteine residues in both the amino and carboxyl terminals, depending on the individual IGFBP. The affinity of an IGFBP for IGF is enhanced by increased phosphorylation of the IGFBP. Only 1% to 5% of the total serum IGF level is free; and 95% to 99% is bound to IGFBP – 90% to IGFBP-3 in a 150-kd complex, and ~5% to IGFBP-1, -2, and -4 as 30- to 50-kd complexes.

The IGFBPs both inhibit and enhance the bioactivity of the IGFs by controlling their clearance from blood and delivery to the target cell. The IGFBPs transport, serve as a reservoir, inhibit the degradation, and consequently prolong the half-life of the IGFs. The 150-kd IGFBP-3/IGF complex has a serum half-life of 14 to 18 hours and is unable to cross the vascular membrane; the half-life of the 40- to 50-kd, capillary permeable IGFBP-IGF complexes is 0.5 hours and that of free IGF is 0.2 hours. The smaller IGFBP-IGF complexes may enhance transcapillary transport of IGF, its delivery to the cell membrane, and interaction with its cellular receptors. The IGFBPs also protect against some of the potential adverse effects of IGF such as hypoglycemia and possibly excessive cell and tissue growth. In addition, the binding proteins have intrinsic biologic activity; thus, IGFBP-1 inhibits DNA synthesis in chick embryo fibroblasts and perhaps ovarian steroidogenesis independently of IGF.¹ IGFBP-3 inhibits insulin as well as IGF-1-mediated growth of IGFBP-3 transfected Balb/c 3T3 fibroblasts.⁴

IGFBP-1

IGFBP-1 is synthesized by human secretory and late proliferative endometrium, placental decidua, granulosa cells, and liver. Near its carboxyl terminal IGFBP-1 has a tripeptide recognition sequence (Arg-Gly-Asp) for the integrins, a group of cell membrane

receptors, suggesting that IGFBP-1 may bind to the cell membrane. IGFBP-1 is present in follicular and amniotic fluid and in human serum, where its concentrations are highest at birth and decline progressively to adolescence. Serum values rise acutely during hypoglycemia and fasting and are high in patients with hypoinsulinemic diabetes mellitus and in those with growth hormone (GH) deficiency. The production of IGFBP-1 is depressed by insulin but stimulated by IGF-1; serum concentrations of IGFBP-1 are inversely related to those of insulin. Insulin also enhances translocation of IGFBP-1 to the extravascular space.⁵ There is a diurnal variation in serum concentrations of IGFBP-1, with highest values at night, reflecting the reciprocal relationship between IGFBP-1 and insulin secretion. Primarily, IGFBP-1 inhibits the metabolic/growth-promoting actions of IGF. It has been suggested that in periods of nutrient deprivation such as hypoglycemia, increased levels of IGFBP-1 bind the IGFs, thus inhibiting their hypoglycemic and anabolic effects and permitting remaining metabolic fuels to maintain or restore cellular energy homeostasis. IGFBP-1 is the major IGFBP synthesized by human granulosa-luteal cells.⁶ It inhibits the proliferative, differentiating, and functional effects of IGF-1 on granulosa-luteal cells, while its production is inhibited by follicle-stimulating hormone (FSH) and IGF-1.⁷ In the polycystic ovary syndrome associated with hyperinsulinism in both lean and obese women, serum concentrations of IGFBP-1 are depressed.⁷ It has been hypothesized that lowered ovarian IGFBP-1 production by hyperinsulinism leads to increased ovarian free IGF-1 and enhanced gonadotropin-mediated androgen production, in turn inhibiting follicular development and leading to follicular atresia.⁵

IGFBP-2

IGFBP-2 is synthesized in the liver, brain, ovary, and endometrium and binds IGF-2 approximately 10-fold more avidly than IGF-1. It too has the recognition sequence (Arg-Gly-Asp) for binding to the cell membrane. Serum concentrations of IGFBP-2 are high in fetal and cord serum and in elderly and GH-deficient patients; they are lowest in pubertal individuals. IGFBP-2 values are increased by administration of insulin and IGF-1.² Serum concentrations of IGFBP-2 are also increased by hypoglycemia, hepatic and renal failure, and leukemia.⁸ IGFBP-2 primarily inhibits IGF action. In contrast to IGFBP-1, IGFBP-2 is synthesized by human granulosa-luteal cells in response to IGF-2 and inhibited by human chorionic gonadotropin.⁹ Serum concentrations of IGFBP-2 are slightly increased in patients with prostate cancer.¹⁰

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IGFBP-3

IGFBP-3 is the major circulating IGFBP; it is associated with an acid-labile glycosylated subunit and with either IGF-1 or IGF-2, a 150-kd complex is formed (Figure 1).¹¹ IGFBP-3 is synthesized in the liver and many other tissues (fibroblasts, ovary, placenta) under the direction of GH. In experimental animal models, serum levels of IGFBP-3 fall after hypophysectomy, but tissue levels of mRNA for IGFBP-3 do not decline, suggesting that the decrease in IGFBP-3 is perhaps due to a decrease in its translation or an increase in its metabolism. In GH-resistant patients, IGF-1 has a biphasic effect on serum IGFBP-3 levels – decreasing values when administered for 7 days but increasing them after 6 months of therapy.^{12,13} Circulating values of IGFBP-3 are constant over a 24-hour period and do not change rapidly in response to metabolic perturbation. IGFBP-3 levels are low in the fetus and neonate and increase throughout childhood; they reach maximum values in midpuberty, are stable during adulthood, and decline with aging. In children and adolescents IGFBP-3 concentrations reflect

the GH secretion rate.¹⁴ IGFBP-3 values are low in GH-deficient subjects and high in hypersomatotropic patients, and are increased by administration of GH. IGFBP-3 both inhibits by sequestering IGF, and enhances the action of IGF by prolonging its half-life, sustaining its delivery to tissue and increasing its cellular binding through a cell surface associated IGFBP-3 molecule, thus perhaps facilitating interaction of IGF with its receptor.¹⁵ Fibroblast-derived cell surface associated IGFBP-3 may be dissociated from the cell membrane by IGF. Transforming growth factor, type β_1 , stimulates transcription of the fibroblast IGFBP-3 gene.¹⁶

The human acid labile subunit of the 150-kd IGF-1 binding complex (Figure 1) is an 84- to 86-kd glycosylated protein of hepatic origin and is composed of 552 amino acids, 22% of which are leucine. It does not bind to IGF, but binds only to IGFBP-3 to which IGF has bound. In serum the acid-labile subunit is present in the free and complexed state. Serum levels of the acid-labile subunit increase between birth and puberty, and decline in older subjects.² Values are high in acromegalic and low in hypsomatotropic patients. GH may directly regulate the synthesis of this material. The biologic role of the acid-labile subunit is uncertain; it may maintain the 150-kd IGF-binding complex, thus decreasing the rate of transfer of IGF with IGFBP-3 to the extravascular space and preventing the hypoglycemic effect of IGF.

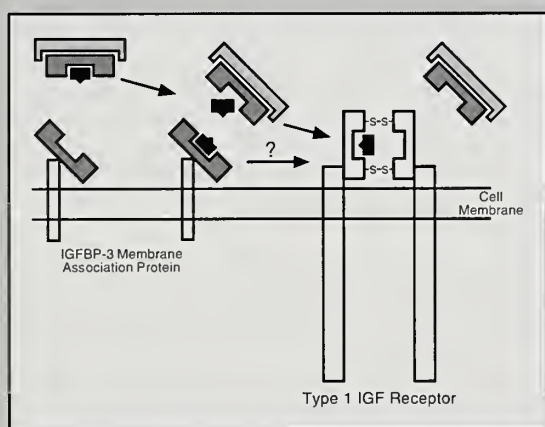
IGFBP-4, IGFBP-5, AND IGFBP-6

IGFBP-4 is synthesized by human fetal and adult liver, brain, ovary, and bone, partly in response to IGF-1.¹⁷ IGFBP-5 has been identified in follicular fluid; it binds IGF-2 severalfold more strongly than IGF-1. IGFBP-6 is synthesized by human adult liver but not by fetal liver, and binds IGF-2 ~70 times more avidly than IGF-1. IGFBP-4, -5, and -6 inhibit IGF-1 and -2 bioactivity.

IGFBP PROTEASES

Serum IGFBP proteases degrade the IGFBPs and alter their binding affinity for and distribution of IGF. The protease for IGFBP-3 is present in the serum of pregnant women and in patients with severe debilitating illness, GH insensitivity, and various neoplastic diseases.⁸ The prostate-specific antigen, a marker of prostatic neoplasia, is the IGFBP-3 protease in seminal plasma.¹² Serum concentrations of IGFBP-3 are decreased in patients with prostate cancer, suggesting that it may have been degraded by its protease, releasing IGF-1 and enhancing the effect of IGF-1 on growth of the neoplasm.¹⁰ IGF-1 does

Figure 1



The circulating 150-kd complex of IGFBP-3 (light gray symbol), acid-labile subunit (dark gray symbol), and IGF (solid symbol) may deliver IGF directly to the IGF receptor or to a membrane-bound molecule of IGFBP-3 (possibly bound to a receptor for IGFBP-3) that, in turn, presents IGF to the IGF receptor.¹⁵ Although each heterodimer of the type 1 IGF receptor can bind one molecule of IGF, the dimerized complex binds but one molecule of GH.

Abbreviations

IGF = insulin-like growth factor
IGFBP = IGF-binding protein

not affect IGFBP-3 protease activity in subjects with GH insensitivity.¹³ A protease specific for IGFBP-4 activated primarily by IGF-2 is produced by adult human fibroblasts.¹⁹ While intact IGFBP-4 inhibits IGF action, the proteolyzed form of IGFBP-4 does not. Thus, the IGFBP proteases may be another important factor regulating IGFBP binding of IGF and the bioavailability of IGF.

OTHER BINDING PROTEINS

A binding protein for IGF-2 distinct from the IGFBPs discussed above has been found in human serum and urine. It has been characterized as the extracellular domain of the IGF-2/mannose-6-phosphate receptor.^{20,21} The physiologic significance of this circulating protein is uncertain.

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Abstracts From the Literature

Influence of the High-Affinity Growth Hormone (GH)-Binding Protein on Plasma Profiles of Free and Bound GH and on the Apparent Half-Life of GH: Modeling Analysis and Clinical Applications

Growth hormone (GH) in serum is approximately equally divided between the free form and that bound to a high-affinity GH-binding protein (GHBP) that is the extracellular portion of the transmembrane GH receptor. Although GH is secreted in an episodic fashion (and it is this pattern of GH release that is most effective in stimulating growth in experimental animals), GHBP provides a circulating reservoir of GH. It also may be important for the presentation of GH to the cellular GH receptor.

Previously, Veldhuis et al demonstrated an apparent inverse relationship between the GH-binding capacity of GHBP and the secretion rate of GH. These workers have now established mathematical models to examine the interaction of GHBP with GH under both equilibrium conditions and in the more physiologic nonequilibrium state. Under equilibrium conditions, as might apply when GH is constantly infused or secreted by some tumors, variation of the secretion rate of constantly infused GH alters the total amount of GH in serum and the amount of bound and free hormone, as well as the percentage of the GHBP occupied by GH. Altering the affinity or capacity of GHBP does not affect the concentration of free GH. Thus, the amount of free GH in serum is directly related to its secretion rate and half-life and inversely related to the volume of distribution of GH, but it is little affected by GHBP itself. The authors' calculations yielded estimated GH half-lives for GHBP-bound GH, free GH, and total GH of 29, 7 to 9, and 18 minutes, respectively.

Applying these mathematical models to the nonequilibrium state, the investigators demonstrated that initially following a pulse of GH secretion, free GH levels increase and then decrease quickly as free GH is removed by both binding GHBP and by catabolic processes. The amount of GH bound to GHBP also increases rapidly, but declines more slowly than do free GH levels. In this system, GHBP can serve as a reservoir of GH potentially available for delivery to target cells during intervals between pulses of GH release.

Veldhuis JD, Johnson ML, Faunt LM, et al. *J Clin Invest* 1993;91:629-641.

Editor's comment: The fact that some protein hormones such as GH and the insulin-like growth factors (IGFs) circulate bound to carrier proteins suggests that these carrier proteins might be physiologically important for the biologic activity of the hormone. That the serum GHBP is the extracellular domain of the GH receptor rather than a nonreceptor-related protein (as are IGF-binding proteins) implies that GHBP may interface with GH at the GH receptor. Perhaps this facilitates contact of GH with its receptor. Since *in vivo* GHBP enhances the growth-promoting effects of GH,¹ it is likely that at least GHBP increases the biologic half-life of GH, although it may well have some more specific effect.

I have observed an occasional hyposomatotropic subject in whom antibodies to exogenous GH have developed, and in whom the linear growth response to GH has remained good to excellent over a period of years without increase in GH dosage. This suggests that in some patients antibodies to GH may also serve to increase the biologic effectiveness of GH.

Allen W. Root, MD

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A Linkage Between DNA Markers on the X Chromosome and Male Sexual Orientation

The relationship between the genetic and environmental components of homosexuality is complex and has not been clearly elucidated. Studies of twins and adoptive siblings have shown the possibility of a large genetic component¹ and the search for a "homosexuality gene" has recently been undertaken. If sexual orientation is somehow programmed by the genes, then homosexuality may finally have a valid scientific explanation.

This recent study by Hamer et al reported linkage analysis of homosexual brothers that suggested there is a gene on the X chromosome that may be responsible for some component of male sexual orientation. The data come from the analysis of 76 homosexual men. The pedigree analysis showed that 13.5% of the gay men's brothers were homosexual; this is much higher than the 2% expected from the general population. It also showed that there were more gay relatives on the maternal side of the family, particularly those uncles and cousins who were sons of maternal aunts. This finding suggested that homosexuality could be a trait passed on through the females and so the possibility of a "gay gene" on the X chromosome.

Random X chromosome markers were identified in 40 pairs of homosexual brothers chosen because there was evidence that homosexuality was being inherited from the maternal side of the family. In order to assess how the markers were inherited, their mother's X chromosomes were also typed. For any X-linked genetic marker, the chance that 2 brothers inherited the same allele from their mother is 0.5. If the brothers inherited the same maternal X-linked markers more frequently than expected by chance, it is thought that they share a trait due to a gene in the region of the shared marker.

The results showed that except for 1 marker on Xq28, all markers along the X chromosome had been inherited as often as expected by chance. At the Xq28 marker, however, 33 of the

40 pairs had inherited the same allele from their mothers. The other 7 pairs of twins were discordant at that marker. Linkage of these markers gave a multipoint lod score of 4.0; this represents a 99.5% chance that there is a gene (or genes) in this area of chromosome X that predisposes to at least one subtype of male homosexuality.

Because Hamer et al did not study the group of homosexual men in whom the trait seems to be inherited from the father, the Xq28 site is not expected to explain all homosexual males. Even if it does, the site of a gene predisposing to male homosexuality may be difficult to find since the Xq28 region of chromosome X is known to contain several hundred unidentified genes.

Needless to say the results of this study will have to be replicated with a different population. If Hamer and colleagues have found the first genetic clue to male homosexuality, it will be interesting to see whether the Xq28 locus plays a role in female homosexuality.

Hamer DH, Hu S, Magnuson VL, et al. *Science* 1993;261:321-327.

Editor's comment: *The possibility that a gene may exist that predisposes to homosexuality is of considerable consequence. It reinforces the possibility that sexual orientation has a genetic basis rather than its being completely determined by the environment. Replication of this study should be easy and may provide further insight into the association between Xq28 and homosexuality.*

Judith G. Hall, MD

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Exclusively Paternal X Chromosomes in a Girl With Short Stature

Uniparental disomy (UPD) occurs when the 2 chromosomes of a pair have been inherited from only 1 parent (Engel, 1980). It is now recognized as a mechanism producing disease with certain chromosomes. Paternal UPD for sex chromosomes in humans has been reported in only 2 cases. The first was a case of paternal disomy of sex chromosomes in a father-to-son transmission of hemophilia A (Vidaud et al, 1989). The second is this recent report by Schinzel et al (1993) documenting paternal UPD for the X chromosome in a girl with a 45,X/46,XX karyotype and some features of Turner syndrome.

The patient reported by Schinzel and colleagues had growth retardation, short neck, broad chest, cubitus valgus, and gonadal dysfunction. Cytogenetic analysis on lymphoblasts showed 45,X/46,XX mosaicism. At age 11 7/12 there were 2/15 cells with a 45,X line; at age 13, 2/25 cells demonstrated a 45,X line, with the other cells being 46,XX; and by age 13 7/12, all 50 cells were 46,XX. DNA analysis on blood showed that both X chromosomes of the 46,XX line were of paternal origin. No other tissue (eg, fibroblasts) had been analyzed at the time of the report.

This report suggests that the most likely mechanism for

paternal XX UPD would be the loss or absence of the maternal X (before, during, or after meiosis) and early postmeiotic non-disjunction with complementation of the paternal X. This would allow for some degree of mosaicism with the 45,X line. It is possible that the phenotypic features of Turner syndrome were due to a higher degree of mosaicism with a predominant 45,X line in other tissues (ie, skin). From the studies in blood it is clear to see that the 46,XX line has a selective advantage; this may not be true for other tissues, in which the predominant line may be 45,X.

New molecular techniques allow the easy recognition of the parental origin of a specific chromosome. Maternal and paternal UPD has been reported for a number of autosomes with different disorders. Maternal UPD of chromosome 15 has been associated with Prader-Willi syndrome (Nicholls et al, 1989), paternal UPD of 15 has been reported with Angelman syndrome (Knoll et al, 1989), and segmental UPD of chromosome 11 (specifically the 11p15 region) has been shown in some cases of Beckwith-Wiedemann syndrome. UPD for chromosomes 22 and 21, however, has no known phenotypic effect (Schinzel et al, 1992).

As mentioned previously, paternal UPD for sex chromosomes has been reported in a few cases (Vidaud et al, 1989; Schinzel et al, 1993). XY mice with paternal UPD for sex chromosomes are known to be normal (Handel et al, 1990). This would suggest that this condition has very few or no phenotypic effects. Maternal XX UPD in female mice has never been reported, but XO female mice survive longer if the single X chromosome is maternally derived (Hunt, 1990). No explanation has been given for the differences in survival. Maternal disomy of the X chromosome in humans has been documented in normal fertile females (Avivi et al, 1992) and does not have any known phenotypic effect.

Maternal UPD for chromosome 7, maternal and paternal UPD for chromosome 16, and now paternal UPD for X chromosome have been associated with growth retardation. The patient reported by Schinzel et al (1993) had growth failure and minimal dysmorphic features suggestive of Turner syndrome. Due to the findings suggestive of Turner syndrome, further study of 8 families with a proband demonstrating growth failure and a 46,XX Turner-like syndrome was undertaken. However, all had

normal paternal and maternal sex chromosome contribution.

It is clear that more research is needed to establish the true incidence of paternal XX UPD and its phenotypic effects. So far the evidence has shown that the X chromosome has very few or no paternally imprinted regions.

Schinzel AA, Robinson WP, Binkert F, et al. *Hum Genet* 1993; 92:175-178.

Editor's comment: *In this particular patient, the short stature may be related to being mosaic for the 45,X Turner syndrome cell line. The question is whether the loss of abnormal cell lines is the usual situation or if 46,XX has sufficient selective advantage to outgrow the 45,X cells. It will be interesting to look at the report of the fibroblast studies in this patient when they are done. They might give a clue to the origin of the 2 cell lines (ie, did the zygote start as 46,XpXp or 45,Xp). The frequency of UPD for sex chromosomes in the "normal" population will be quite interesting to determine.*

Judith G. Hall, MD

In Vivo Gene Therapy of Hemophilia B: Sustained Partial Correction in Factor IX Deficient Dogs

Hemophilia B is an X-linked clotting disorder affecting about 1 in 30,000 males. It can be managed successfully by infusion of virus-free factor IX preparations; however, the high cost of such preparations has limited their use and has led investigators to search for alternative therapies. This report from Savio Woo's team in Houston provides insight into potential gene therapy for this condition.

The investigators had previously demonstrated that retroviruses could successfully be used to deliver recombinant genes to liver cells in mice. In this study they extended their work to larger animals, targeting hepatocytes in dogs manifesting hemophilia B.

First, to show that a foreign gene could be transferred successfully to canine liver cells, they infused the portal veins of normal dogs with a retroviral vector containing the *Escherichia coli* β -galactosidase gene. Analysis of liver tissue and cells 2 weeks later revealed enzyme activity in some cells. Since this enzyme is not normally present in mammalian cells, its presence in this instance indicates that gene transfer was successful.

Next, they prepared a retroviral vector containing the coding sequences (cDNA) of canine factor IX and infused it into the portal veins of 3 hemophilia B dogs. The dogs harbored a missense mutation in the catalytic domain of the factor IX gene that abolished the antigenicity and severely disturbed the function of the clotting factor. The animals were then monitored for up to 9 months by immunoassays of factor IX antigen and by functional assays for the intrinsic pathway for clotting in which factor IX participates.

Plasma factor IX levels rose from undetectable to 2 to 10 ng/mL, where they remained for 6 months in 1 dog and for 9 months in another. More importantly, whole blood clotting times ranged from 15 to 25 minutes after treatment, compared with 45 to 55 minute values for untreated factor IX deficient littermates. Normal values for dogs range from 6 to 8 minutes.

Similarly, partial thromboplastin times decreased substantially after treatment, compared with pretreatment values. The improvement in clotting assay times continued through the follow-up period as noted above.

The authors acknowledge that the factor IX levels achieved by gene transfer were only about 0.1% of normal. However, they emphasize that these levels resulted in a substantial improvement in standard clotting parameters. They feel that the results demonstrate the feasibility of in vivo retroviral-mediated gene transfer into the livers of large animals. With technical advances in vector construction, they foresee potential uses of this approach for treatment of hemophilia B and other metabolic disorders secondary to hepatic deficiencies in humans.

Kay MA, Rothenberg S, Landen CN, et al. *Science* 1993;262: 117-119.

Editor's comment: *It is ironic that when gene therapy was first contemplated, many wondered how the genes of interest would be assembled, what regulatory elements would be used to control their expression, and how the regulatory elements would be incorporated into vectors. Although construction of vectors that permit therapeutic genes to be expressed appropriately is still a formidable task, it is clear that delivery of such vectors to where they are needed (ie, cells, tissues, organs, etc) is also a major problem – perhaps an even bigger challenge. In other words, the greatest obstacle to gene therapy may lie in its cell biology rather than in its molecular biology. This paper reflects the recent attention that the delivery aspects of gene therapy are beginning to receive, which in turn reflects the rapid evolution of this field.*

William A. Horton, MD

Molecular Basis of the *Little* Mouse Phenotype and Implications for Cell Type-Specific Growth

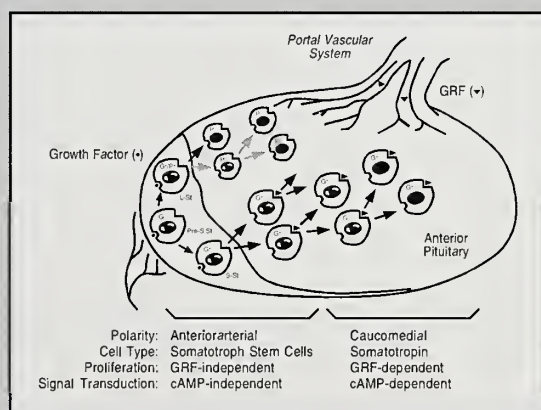
The investigators demonstrate that in the growth hormone (GH)-deficient *little* mouse the somatotrope receptor for GH-releasing hormone (GHRHr) is abnormal. There is substitution of adenine by guanine (A→G) in the second nucleotide of codon 60 resulting in replacement of a highly conserved aspartic acid by glycine (Asp 60→Gly) in the amino terminal, extracellular, ligand-binding domain of the GHRHr. GHRH was unable to stimulate intracellular production of cyclic adenosine monophosphate in a CV1 cell line transfected with and expressing the abnormal GHRHr, whereas expression of wild-type GHRHr in these cells revealed normal GHRH activity, consistent with a functionally defective GHRHr in the *little* mouse. Examination of the microscopic structure of the anterior pituitary of these animals revealed hypoplasia and substantial loss of GHRHr and GH-expressing somatotropes but a normal population of lactotropes. Those pituitary cells expressing GHRHr and GH were confined to the periphery of the anterolateral regions of the adult gland. (In the normal mouse pituitary gland GHRHr and GH-expressing cells are widespread and generally distributed.) Pit-1, a transcription factor necessary for differentiation and function of somatotropes and other cells, was normally expressed in the pituitary of the *little* mouse. The authors conclude that Pit-1 is important for initial differentiation of stem cells and for differentiation of the common somatomammotrope into lactotropes and somatotropes, and that GHRH (requiring normal GHRHr) is responsible for further differentiation, distribution, and function of the mature GH-producing cell. Thus, there would appear to be a reservoir of somatotropes that, under the guidance of GHRH, divide and migrate to the caudomedial region of the normal mature anterior pituitary gland.

Lin S-C, Lin CR, Gukovsky I, et al. *Nature* 1993;364:208-213.

Editor's comment: *Pit-1 is a tissue-specific POU domain protein that serves as a transcription-activating factor for genes, leading to differentiation of somatotropes, lactotropes, and thyrotropes, and for expression of the GH and prolactin genes. Absence of a functional Pit-1 protein (in the Snell rat and in humans) leads to pituitary hypoplasia and deficiencies of GH, prolactin, and thyrotropin secretion. The present article offers good evidence to suggest that after initial differentiation of stem cells, GHRH (and normal GHRHr) is required for further differentiation and function of the somatotrope. The list of possible causes of GH deficiency grows longer and now includes defective GHRHr. One suspects that human examples of the little mouse animal model of hypopituitarism will be detected.*

Allen W. Root, MD

Figure 1



Pit-1 Mediated GRF Regulation of Somatotrope Proliferation Model of growth hormone-releasing factor (GRF) receptor modulation of somatotrope proliferation lineage. A presomatotrope stem cell (Pre-S St) is proposed to give rise to either a somatotrope-stem cell (S St) or lactotrope-stem cell (L-St) that at some point must express both growth hormone (GH) and prolactin (G⁺, P⁺). Somatotrope stem cells (S St) express GH (G⁺), and at least a subset are GRF-receptor positive (Y). Stem cells are located in the anterolateral circumference of the gland. Caudomedial proliferation of somatotropes requires GRF receptor and GRF, which is supplied by the portal circulation from median eminence. Somatotropes proliferate (indicated by mitotic spindle) across the entire anterior pituitary. In contrast, lactotropes, once differentiated, proliferate little, if at all (gray arrows and nucleus). The 2 zones of proliferation, representing stem cells or mature somatotropes, are cyclic adenosine monophosphate (cAMP)-independent or cAMP-dependent, respectively.

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In Future Issues

Insulin-Like Growth Factor 2 and Growth by Yves Le Bouc, MD

Osteochondrodysplasias With Mild Clinical Manifestations: A Clinician's Guide by Richard M. Pauli, MD

Noonan Syndrome: A Review by Michael A. Patton, MA, MSc, MD

Prader-Willi Syndrome: The Unfolding Genetic Story by Uta Francke, MD

Treatment of Craniopharyngioma: End Results by Edward Laws, MD

Overexpression of Dystrophin in Transgenic *mdx* Mice Eliminates Dystrophic Symptoms Without Toxicity

The development of gene therapy for human diseases has been gathering momentum on many fronts. Cox and colleagues cite a good example by reporting correction of many of the pathologic manifestations in a mouse model of Duchenne muscular dystrophy (DMD).

The investigators studied the *mdx* mouse, a mouse strain with a mutation in the dystrophin gene that eliminates expression of the muscle and brain forms of the protein. Although *mdx* mice do not exhibit the severe muscle weakness typical of DMD, they manifest marked impairment of diaphragm function and progressive degeneration and fibrosis of muscle fibers similar to that found in DMD.

Cox et al introduced a dystrophin transgene into *mdx* mice using standard microinjection techniques. The transgene contained the full-length mouse dystrophin cDNA controlled in a tissue-specific manner by regulatory elements of the mouse creatine kinase gene to obtain expression in muscle. Extensive comparisons were made between control mice, *mdx* (nontransgenic) mice, and *mdx* transgenic mice.

Several assays revealed expression of dystrophin in transgenic *mdx* muscle tissues. Immunoblot (western) analysis identified a skeletal and cardiac muscle-specific protein of appropriate size for dystrophin in transgenic *mdx* muscle but not in muscles from nontransgenic *mdx* mice. The protein was present in amounts about 50 times normal. Immunostaining confirmed these findings and localized the dystrophin to its normal site, the sarcolemma, as seen in control but not *mdx* mice. Histologic examination of muscles showed that the degenerative changes seen in the *mdx* mouse were virtually absent from the transgenic *mdx* muscles.

The investigators also examined the functional effects of the dystrophin transgene expression. Analysis of the contractile properties of the diaphragm revealed no differences between control and transgenic *mdx* muscle, whereas the power of *mdx* diaphragm to contract was reduced about one third from normal. Normally, dystrophin is associated with and induces the synthesis of sarcolemmal glycoproteins called dystrophin-associated proteins. These proteins are absent from DMD and *mdx* muscle; however, they were detected in transgenic *mdx* muscle.

Thus, the authors demonstrated correction of many of the pathologic markers and the functional sequelae of the *mdx* mutation without causing obvious harm to the mice. As emphasized by the investigators and in an accompanying editorial by Blau, one of the more interesting observations is that the considerable overexpression of apparently normal dystrophin did not appear to have deleterious effects on muscle structure or function.

Cox GA, Cole NM, Matsumura K, et al. *Nature* 1993;364:725-729.
Blau HM. *Nature* 1993;364:673-675.

Editor's comment: A long-term concern of potential gene therapists has been that genes introduced therapeutically must be precisely regulated to be effective and safe. This suggested that entire genes, including their intervening sequences and upstream and downstream regulatory elements, may need to be packaged in therapeutic vectors. Such restrictions would eliminate many large genes, such as the dystrophin gene, from consideration for commonly used vectors, such as retroviruses, on the basis of size alone.

This paper reports that coding sequences alone, controlled by a promoter different from its own, can produce a tissue-specific functional protein that interrupts the natural course of the disease. This is technically a very important accomplishment. Surprising was the fact that the very large amount of gene product was still effective. As acknowledged by both Cox et al and Blau, this raises the possibility that regulation of "artificial" genes introduced for treatment may not need to be as tight as once believed, at least in some instances.

The strategy used to introduce the dystrophin cDNA in this investigation is obviously not suitable for humans. Nevertheless, the observations are quite relevant and will add to the growing body of knowledge needed to apply gene therapy to humans.

William A. Horton, MD

The Y Chromosome in Turner Syndrome

The most common (40% to 60%) chromosomal constitution in patients with Turner syndrome is 45,X. With a 45,X karyotype in Turner syndrome it was initially thought that all patients lacked the entire second sex chromosome (either X or Y). However, the high lethality of a "pure" 45,X karyotype has led to the suggestion that most, if not all, live-born 45,X Turner patients are mosaic 45,X/45,XY or 45,X/46,XX.

The main functions of the mammalian Y chromosome are sex determination, early sexual differentiation, and the control of spermatogenesis and spermiogenesis. Recent DNA molecular studies have shown that some patients with 45,X – and even mosaic 45,X/46,XX – have residual cytogenetically undetectable Y chromosomal material in blood.^{1,2} Further studies have shown that although the Y chromosome material may not be present in

peripheral blood, there is a probability of its being present in skin.

Some Turner syndrome patients have residual testicular tissue, and if there is some degree of mosaicism for Y chromosome DNA sequences, patients may experience excessive virilization and may be at increased risk for gonadoblastoma. This is why it is important to use reliable and sensitive diagnostic methods to ascertain the presence of Y chromosome material in Turner syndrome.

The study by Kocova et al evaluated 18 patients with clinical features of Turner syndrome. Ten patients had a 45,X karyotype, 7 were mosaic with a numeric or structural anomaly of the X chromosome, and 1 was 45,X/46,XX. None of these patients had any evidence of Y chromosome material in blood cytogenetic studies.

Polymerase chain reaction (PCR) and Southern blot techniques were used to detect the sex-determining region (SRY gene) and a repetitive sequence DYZ3 near the centromere of the Y chromosome in blood cells. Their results showed that 6 of 18 patients had positive Y chromosome signals. Three were readily detected on Southern blot and the other 3 showed chromosome Y material only after PCR amplification. They concluded that a "pure" 45,X karyotype is probably less common than is usually reported on cytogenetic studies and that Y mosaicism may go unnoticed unless PCR amplification is done.

Previous investigators¹ have been successful in using Southern blot to detect Y chromosome material. PCR amplification is a very sensitive method, and it is a more accurate method for determining the presence of Y sequences that may not be present in high-resolution karyotyping. Because of its high sensitivity, this method significantly increases the ability to look for micromosaicism in lymphocytes or skin fibroblasts.

The combination of Southern blotting and PCR techniques is helpful in identifying Y chromosome sequences in patients with Turner syndrome. These techniques may be required for some Turner syndrome patients with monosomy X or mosaic karyotype who have residual testicular tissue X. This will enable us to offer better counseling and clinical management.

Kocova M, Siegel SF, Wenger, SL, et al. Detection of Y chromosome sequences in Turner's syndrome by Southern blot analysis of amplified DNA. *Lancet* 1993;342:140-143.

Editor's comment: In view of this evidence, the present challenge for practitioners is in deciding how far to go in looking

for Y chromosome material. PCR is obviously a helpful tool; however, it is important to remember that blood may not be enough to rule out mosaicism. We may need to look at fibroblasts. It may be necessary to establish new guidelines for managing Turner patients at risk of malignancies.

Judith G. Hall, MD

1. Muller U, et al. *Hum Genet* 1987;75:109-113.
2. Gemmill RM, et al. *Am J Hum Genet* 1987;41:157-167.

2nd Editor's comment: Approximately 50% of patients with Turner syndrome have the 45,X karyotype; the remainder have either X chromosome mosaicism and/or abnormal X chromosome formation. Since the majority of 45,X conceptuses abort spontaneously, it has been suggested that many of the surviving 45,X patients may have subtle mosaicism. The present report demonstrates that many patients with the Turner phenotype carry the SRY sequence, but the clinical relevance of this observation is as yet unknown. As Held (*Lancet* 1993;342:128-129) suggests, the development of a 45,X-SRY+ subject could be the consequence of XY interchange followed by loss of 1 X chromosome by anaphase lag. Since the presence of Y chromosomal material places the Turner syndrome patient at increased risk for gonadoblastoma, he recommends that, when indicated, initial search for a Y chromosome fragment not detectable cytogenetically be conducted by fluorescence in situ hybridization (FISH) until the methodology of Kocova et al has been validated and the significance of the finding ascertained.

Allen W. Root, MD

A Nonpeptidyl Growth Hormone Secretagogue and Stimulation of Growth Hormone Release From Rat Pituitary Cells by L-692,429, a Novel Non-Peptidyl GH Secretagogue

The investigators describe a benzolactam derivative chemically engineered by analysis of the structure-activity relationships of the growth hormone-releasing hexapeptide (GHRP-6 = His-D-Trp-Ala-Trp-D-Phe-NH₂) that suggested that the aromatic amino acids and the NH₂-terminal amine were the important bioactive sites of this molecule. The active R enantiomer, designated L-692,429, functions in a manner similar to GHRP-6. First, as does GHRP-6, it has a synergistic effect with growth hormone-releasing hormone (GHRH) on GH release and intracellular levels of cyclic adenosine 3',5'-monophosphate (cAMP). Second, it has no added effect on GH release when paired with maximal amounts of GHRP-6. Third, its effect on GH secretion is antagonized by an inhibitor of GHRP-6 activity, and the effect of GHRP-6 is inhibited by an antagonist of L-692,429. Fourth, rat pituitary cells desensitized to either GHRP-6 or L-692,429 are insensitive to the GH-releasing effect of the other compound but not to GHRH. Fifth, both GHRP-6 and L-692,429 block potassium currents and increase intracellular calcium levels, leading to depolarization of pituitary cells and GH secretion. Sixth, both GHRP-6 and L-692,429 activate protein kinase C. When administered intravenously, L-692,429 stimulates GH release in primates (including humans) and in rats, dogs, pigs, and sheep. Serum concentrations of corticotropin and cortisol

increase slightly after its administration, but serum levels of luteinizing hormone, prolactin, insulin, and thyroxine do not change. Somatostatin inhibits the effect of L-692,429 by preventing depolarization of the pituitary cell.

Smith RG, Cheng K, Schoen WR, et al. *Science* 1993;260:1640-1643.

Cheng K, Chan WW-S, Butler B, et al. *Endocrinology* 1993;132:2729-2731.

Editor's comment: The authors have introduced a nonpeptidyl compound that is likely to be absorbed from the gastrointestinal tract without digestion/degradation; thus it is orally active, permitting development of an enteral therapeutic agent. Furthermore, these data complement those implied by the identification of the specific GH-releasing property of GHRP-6 (an analogue of enkephalin, but one devoid of opioid activity) regarding the presence of an endogenous ligand with GH-releasing activity distinct from GHRH that has yet to be identified. Analysis of basic structure-function relationships is likely to have again proven valuable in the development of clinically therapeutic agent(s).

Allen W. Root, MD

Influence of Spontaneous or Induced Puberty on the Growth Promoting Effect of Treatment With Growth Hormone in Girls With Turner's Syndrome

Among 36 girls with Turner syndrome treated for 3 years with recombinant human growth hormone (rhGH) 1 IU/kg/wk in daily subcutaneous injections, 15 (aged 9.2 ± 2.2 years) remained prepubertal during the 3 years of treatment, 4 (aged 12.0 ± 1.2 years) showed spontaneous breast development, and 17 (aged 12.9 ± 1.7 years) received ethinyl estradiol 0.1 $\mu\text{g/kg/d}$ during the third year on rhGH.

Height velocity increased significantly during the first rhGH treatment year in all patients, then decreased but remained above baseline values. Height velocity was higher in the few patients who developed spontaneous puberty than in age-matched controls: 8.9 ± 1.2 cm/y vs 7.4 ± 1.2 cm/y, $P < 0.05$. Estradiol 0.1 $\mu\text{g/kg/d}$ seemed to significantly reduce the growth velocity during the third year: 4.0 ± 1.6 cm/y vs 5.6 ± 1.2 cm/y in the nonpubertal patients. However, this difference related mainly to age, since the growth rate expressed in standard deviation score (SDS) for age was not different in the 2 groups: 2.2 ± 1.3 vs 2.2 ± 1.0 SDS.

Multivariate regression analysis showed some discrepancies – mainly a positive effect of estradiol on apparent bone maturation in patients receiving it during the second year of treatment but not during the third year.

The authors conclude: "The onset of spontaneous puberty during the first years of rhGH treatment seems to have an additive effect to rhGH on height velocity. Induction of puberty with oral administration of 100 ng/kg/d ethinyl oestradiol did not

have any beneficial effect on height velocity and seems therefore not to be the optimal way to induce puberty with an adequate pubertal growth spurt in girls with Turner's syndrome under rhGH therapy. Different doses and routes of oestrogen administration have to be evaluated in order to mimic the growth promoting effect of spontaneous puberty as well as possible."

Massa G, Maes M, Heinrichs C, et al. *Clin Endocrinol* 1993;38: 253-260.

Editor's comment: Among several recent reports of the possible effects of associating estrogens with GH in Turner syndrome at the age of physiologic puberty, this one seems particularly relevant. Our own data (not yet published) are in good accordance with those of the present authors: a very low dose of estradiol given at an age of approximately 12 years, or rather at a bone age close to 11 years, has no significant positive or negative effects on the growth rate obtained with GH. At a higher dose, or given earlier, estradiol could reduce the growth rate and/or accelerate bone maturation. The positive effect of spontaneous sexual development on growth rate in Turner girls, documented in a small number of patients in the present study, seemed less certain in our data, and will have to be established with larger groups of patients compared with controls.

Jean-Claude Job, MD

Hazards of Pharmacological Tests of Growth Hormone Secretion in Childhood

Three patients underwent growth hormone (GH) stimulation tests with insulin-induced hypoglycemia and developed severe complications. Two died and 1 sustained neurologic damage.

A 4½-year-old girl was tested with 0.1 U/kg of insulin given intravenously. Nausea, sweating, and tachycardia occurred within 35 minutes, and she became unresponsive. She was given 50% dextrose and 100 mg hydrocortisone intravenously, and 1 mg of glucagon intramuscularly. Blood glucose measured for the first time 1 hour after insulin was greater than 44 mmol/L. Generalized convulsions occurred, which were controlled with IV diazepam and phenytoin. The blood glucose increased to 130 mmol/L and plasma osmolality rose to 388 mmol/L. Treatment with IV insulin was given, and plasma osmolality fell to 339 mmol/L. She became hypotensive and acidotic (arterial pH of 7.02), and then comatose with fixed dilated pupils and no brain-stem reflexes. Cerebral edema was demonstrated by computed tomography (CT). No definite cortical activity was noted by electroencephalography (EEG). Renal failure with anuria ensued, and death occurred 24 hours after the start of the test.

A 9-year-old girl with developmental delay was admitted for investigation of short stature and polyuria. The septum pellucidum was absent on the CT scan, consistent with de Morsier syndrome. Insulin (0.1 U/kg), thyrotropin-releasing hormone, and luteinizing hormone were given IV. At 20 minutes, her blood glucose concentration was 2.0 mmol/L, and the patient became drowsy. With IV dextrose and hydrocortisone, her blood

glucose increased to 17 mmol/L, but she developed generalized convulsions, which were treated with diazepam, paraldehyde, phenytoin, and thiopentone. Two hours post insulin, the plasma sodium concentration was 110 mmol/L and her potassium concentration was 2.7 mmol/L. Repeat CT scans revealed a right-sided subdural hematoma with a shift of the midline. Subsequent CT scans revealed considerable cerebral edema with tentorial herniation and intracerebral hemorrhages. Diabetes insipidus responded to desmopressin treatment. The patient recovered, but seizures remained a problem.

The third patient, a 2-year-old girl, was admitted to investigate short stature and secondary hypothyroidism. After an overnight fast, glucagon 100 $\mu\text{g/kg}$ was given IM. Three hours later, she ate a partial lunch, and was awake and crying at discharge. However, plasma glucose concentrations after the administration of glucagon were later found to be 0.5 to 1.0 mmol/L. At home, vomiting began; she progressively deteriorated and became unconscious. In a local emergency department, her blood glucose concentration was 1.0 mmol/L. External cardiac massage was given, and she was intubated and treated with intravenous bicarbonate, adrenaline, hydrocortisone, and dextrose. When transferred to a referral center, she became hypotensive with fixed dilated pupils, and the EEG showed total electrocerebral inactivity. A small pituitary gland and an atrophic thyroid gland were found at postmortem examination. The tolerance test revealed deficiencies of thyrotropin-stimulating hormone and GH. Cortisol secretion was normal.

The authors concluded that given the risks of pharmacologic testing for GH deficiency, these tests should be avoided; the selection of patients for GH treatment should not be determined by GH response to a pharmacologic test but by the growth rate of the patient before and during treatment.

Shah A, Stanhope R, Matthew D. *Br Med J* 1992;304:173-174.

Editor's comment: In this paper, 3 cases with major tragic complications associated with pharmacologic diagnostic tests for GH stimulation were presented. This paper is reported now, although it was published in 1992, because it encourages all endocrinologists to exercise caution while performing stimulation tests, and prompts us to reconsider the validity of such assessments. As was clearly presented in the paper, the complications described were preventable with appropriate management; they were not solely due to the intrinsic risks of the test. Two patients developed hyperglycemic hyperosmolar coma, in addition to complications, as a result of their management after the test, and 1 developed severe hypoglycemia that was not recognized nor treated properly.

In this era of the "gatekeeper" in medical care, this paper emphasizes that only experienced and qualified individuals should perform provocative stimulation tests for diagnosing GH deficiency. They should also be qualified to effectively manage the possible complications.

These tests should be performed in a hospital setting, and with qualified personnel and the equipment necessary to treat unexpected episodes such as hypoglycemia. Blood glucose

should be monitored by a glucometer throughout the test. Finally, overtreatment should be avoided. The dose of glucose infused when hypoglycemia ensues should be 0.5 to 1.0 g/kg. This amount of glucose should be infused even when no adverse effects are seen at the end of the test. This ensures rapid restoration of blood glucose in case there is a delay in oral intake.

Regarding the validity of the instruments used for diagnosis of short stature children, it is worth questioning the necessity of potentially risky tests. These tests may not necessarily qualify the condition of the patient to a more precise degree, nor do they determine the treatment to be used any better than less risky tests. The so-called gold standard for assessment of GH deficiency—the insulin tolerance test—needs to be challenged, not only because it may cause harm but also because it may yield inappropriate information. Throughout the country, pediatric endocrinologists waiver on the levels of GH elicited by insulin-induced hypoglycemia to diagnose hypopituitarism: is it more than 7, 8, or 10 mg/dL of serum GH? What is the minimal response needed to avoid GH treatment? Do the levels achieved after pharmacologic testing determine the response to treatment? Isn't it better to make such decisions based on accurate physical measurements and monitoring and assessment of the growth rate of short children?

In this era of cost containment, pediatric endocrinologists need to reexamine the validity of their procedures and be sure that we continue to do what we have always done best: treat patients, not test results.

Fima Lifshitz, MD

Life With Turner's Syndrome: A Psychosocial Report From 22 Middle-Aged Women

Sylvén and colleagues utilized a semistructured interview to assess social functioning, emotional development, sexuality, and coping style in a group of 22 middle-aged women with a median age of 44.5 years (range, 39-63 years) with Turner syndrome. These individuals included 10 (45.5%) with a 45,X karyotype and 12 (54.5%) who were mosaic. The interview included questions concerning family background, social identity, emotional development, relationships, female identity, sexuality, and Turner syndrome. The median age at diagnosis was 17.5 years. The majority (12) were diagnosed when puberty failed to occur. Complaints of short stature prompted 7 individuals to seek medical care. Seventeen (77%) of the women reported good contact with their parents and had left home at an average age of 19 years to pursue work, studies, or to marry. All completed elementary school, but only 3 had completed upper secondary schools. Work was considered important for self-esteem and self-confidence, and no woman had been unemployed for a long period. Six were unable to attain desired employment because of their body height, including 1 woman who was not admitted to a nursing school because of her height. Three stated that their parents had suggested they not pursue a physically demanding career. Nineteen (86%) of the women felt the need for improved self-esteem, and of the 16 (72.7%) that had suffered from depression, half (50%) cited infertility as the key factor. For these women, the most important relationship was with their mothers. Ten of the 22 had few friends during their school years, even though 17 presently have very close friendships. Female identity was defined by most in

terms of being in a relationship, but many felt that something was missing psychologically and in their appearance. The median age at which female hormone replacement therapy was begun was 18 years. This may have contributed to the lack of friends and isolation from peers during their school years. Thirteen of the 22 stated that menstruation was a positive experience but unfortunately reminded them of their infertility. During their teenage years all 22 experienced romantic fantasies. Fifteen were presently married, and their median age at first sexual experience was 19.5 years. Eleven (50%) of these adult women were reasonably satisfied with their body; however, 12 (54%) stated that short stature had been psychologically

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upsetting to them. Their first thought at diagnosis usually concerned infertility, and this was the most painful aspect of the diagnosis. Thirteen women (60%) felt that they had received insufficient information concerning their diagnosis from their health-care team. Nineteen of the 22 women (86%) were satisfied with their present life.

In analyzing their findings, the authors identified 4 different ways of coping. Seven of the women were judged to act in an adolescent manner. An additional 7 expressed a chronic feeling of inferiority. Four expressed grief that they had not been able to discuss their diagnosis. Four women were felt to be unaffected by their disorder. The authors point out that these women had been deprived of important psychosocial development and maturation, and many did not understand the significance of their diagnosis.

Sylvén L, Magnusson C, Hagenfeldt K, et al. *Acta Endocrinol* 1993;129:188-194.

Editor's comment: This is an interesting report. There are few data available on the lifetime adjustment of middle-aged women with Turner syndrome. Most psychosocial studies have been performed with relatively young women. Unfortunately, the authors failed to interview a control group of similarly aged women, and it may be that much of the data reported by these women would be similar to those of other women without chromosomal disorders. Unfortunately, these women were diagnosed at a relatively late age, and, therefore, hormone replacement therapy was begun at an age when most women would have been completing their formal education. Growth hormone therapy was obviously not available to improve their stature 27 years ago. One can only wonder whether earlier treatment of short stature and appropriately timed hormonal replacement therapy will alter the adult psychosocial status of girls currently being diagnosed with Turner syndrome.

William L. Clarke, MD

Final Height in Patients Treated for Childhood Acute Lymphoblastic Leukemia

Sklar et al determined the adult (females >18 years; males >21 years) heights of 127 patients treated (in 4 institutions) before 12 years of age for acute lymphoblastic leukemia (ALL) who were disease free, had received no growth-stimulating therapy, and had undergone spontaneous sexual maturation. Various chemotherapeutic regimens were utilized in these patients, but the type and intensity of chemotherapy did not affect final height. Growth data in this patient population were analyzed based on exposure to irradiation of the central nervous system (CNS-XRT). The mean (\pm SEM) height standard deviation score (SDS) at diagnosis was within the normal range in all groups ($+0.28 \pm 0.12$). Height SDS declined in all subjects during treatment, but final height SDS was less than height SDS at diagnosis in 75% of patients. In all groups of patients, adult stature SDS was significantly less ($P < 0.01$) than the height SDS at diagnosis:

Patient Groups	n	Adult Height SDS
Chemotherapy alone	38	0.49 ± 0.14
1800 cGY CNS-XRT	36	0.65 ± 0.15
2400 cGY CNS-XRT	53	1.38 ± 0.16

Patients receiving CNS-XRT lost more height than those who did not, and the higher the dose of CNS-XRT the greater the loss of final height. Much of the loss of adult stature was recorded in females ≤ 4 years of age at diagnosis who also received CNS-XRT (1800 cGY, -1.38 SDS; 2400 cGY, -2.68 SDS), attributable in part to early pubertal development and partial deficiency of growth hormone secretion in these subjects, although no data concerning these phenomena were reported.

As reported by Katz et al, the Pediatric Oncology Group (POG) has gathered data on adult stature (females >16 years; males >18 years) in 109 subjects treated in 2 similar multiarm POG chemotherapeutic protocols for children with ALL, where 51 patients also received 2400 cGY cranial irradiation and 58 patients received no XRT. All of the patients entered puberty spontaneously, and none received growth hormone (GH) therapy. Mean height SDS at diagnosis was -0.06 ± 1.4 .

The adult stature of subjects receiving only chemotherapy was -0.14 SDS, while the final height of the group who also received CNS-XRT was -1.04 SDS ($P < 0.001$). Final height SDS was lower in female patients than in male patients, whether or not they had also received CNS-XRT.

Sklar C, Mertens A, Walter A, et al. Final height after treatment for childhood acute lymphoblastic leukemia: comparison of no cranial irradiation with 1800 and 2400 centigrays of cranial irradiation. *J Pediatr* 1993;123(1):59-64.

Katz JA, Pollack BH, Jacaruso D, et al. Final attained height in patients successfully treated for childhood acute lymphoblastic leukemia. *J Pediatr* 1993;123(4):546-552.

Editor's comment: The adverse effect of CNS-XRT on linear growth in children in the first several years after treatment has been long recognized, but these 2 articles now report long-term, final height data in 246 subjects. In children with ALL not receiving CNS-XRT, growth may be somewhat compromised (although the 2 studies disagree on this point), primarily due to the severity of the illness, its treatment (often with glucocorticoids), and inanition. However, once therapy is completed further loss in height does not occur (Sklar et al). Similarly for children receiving 1800 cGY CNS-XRT, there is no incremental height loss after completion of therapy. However, in children receiving 2400 cGY CNS-XRT, adult height may be substantially compromised (due both to partial deficiency of GH and to early or precocious puberty), although most children will have an adult stature within ± 2 SD of the mean.

Two groups have reported data on the growth of children undergoing bone marrow transplantation (BMT) and total body irradiation (TBI). Bozzola et al¹ administered 1200 cGY TBI (in 6 fractions over 3 days) to 18 children whose growth rates were normal prior to therapy. They observed an immediate decline in growth rate in 9 children who had received prior CNS-XRT (1800 cGY); growth rate remained normal in 9 children who had not received CNS-XRT for the first 2 years after BMT, but declined in the third year. Sixteen of the 18 patients had subnormal GH secretion (peak < 10 ng/dL) by provocative testing. Five of 7

subjects treated with GH responded with an increase in growth velocity. Thomas *et al*² compared the growth of 66 children undergoing BMT, preconditioned with TBI administered as a single dose (900 to 1000 cGy) or in fractions over 3 days (1200 cGy in 6 fractions; 1440 cGy in 8 fractions). Height SDSs prior to BMT were near zero (as were midparental heights). In patients receiving a single TBI fraction, growth rate fell immediately after BMT and height SDS continued to decline over the next 3 years (-0.90 ± 0.90). In children undergoing fractionated TBI, growth velocity declined less rapidly; however, 3 years after BMT, height SDS was significantly lower (-0.22 ± 1.02) than it had been before BMT. Children who received CNS-XRT prior to BMT had a more profound decline in growth rate with either TBI regimen. Patients who received only fractionated TBI did not lose significant height within 3 years after BMT, whereas those without prior CNS-XRT who received single-dose TBI did lose substantial height (SDS, -0.74 ± 1.1). The growth in sitting height was more impaired by TBI (no matter how fractionated) than was growth in leg length, suggesting that the adverse effects of TBI on vertebral epiphyses may be more profound

than on the femoral and tibial epiphyses. In this study, 17 children received GH, with no significant effect on height SDS over 3 years of therapy.

These reports lead to the following conclusions: (1) Growth can be impaired by ALL and its primary treatment; (2) CNS-XRT at a dose of 1800 cGy impairs growth acutely but results in only slightly more loss of adult stature than does the primary disease and its treatment; (3) TBI delivered in fractionated doses does not impair growth (for 3 years after BMT); (4) CNS-XRT at a dose of 1800 cGy followed by TBI for BMT leads to further growth retardation; (5) CNS-XRT at a dose of 2400 cGy significantly impairs growth; (6) TBI impairs vertebral growth to a greater extent than limb growth; (7) females receiving CNS-XRT lose significantly more adult height than do males; and (8) the efficacy of GH therapy in children with BMT is questionable.

Allen W. Root, MD

1. Bozzola M, *et al.* *Horm Res* 1993;39:122-126.

2. Thomas BC, *et al.* *Eur J Pediatr* 1993;152:888-892.

Growth and Growth Hormone Secretion After Bone Marrow Transplantation

Growth and its hormonal factors were studied in 29 children (14 males, 15 females) having undergone bone marrow transplantation (BMT) prepared according to 4 different protocols: total body irradiation (TBI) of 10 grays (Gy) in a single exposure (group 1, 11 children aged 7.3 ± 1 years); TBI of 8 Gy in a single exposure (group 2, 4 children aged 3.1 ± 0.6 years); TBI of 12 Gy given as 6 fractionated doses (group 3, 7 children aged 5.3 ± 0.2 years); or chemotherapy alone (group 4, 7 children aged 1.3 ± 0.4 years). Growth hormone (GH) secretion was first evaluated, 2 to 7.5 years after transplantation, by combined arginine-insulin stimulation test, with a peak above 10 ng/mL in 26 patients, and 6.9 to 8.9 ng/mL in 3 patients of group 1. A second evaluation was performed 2 to 5 years later in 10 patients, with normal results in 8, a subnormal GH peak value in 2 patients from group 1, but no significant change from the first evaluation, and no deficiency of the nocturnal GH plasma levels. Plasma IGF-1, at the time of first evaluation, was normal for sex and age in 18 patients, subnormal in 11, including 2 low GH-responders, and not correlated with the body mass index. At the time of the second evaluation, the mean plasma IGF-1 level was not significantly changed, but 3 of the 4 patients having low IGF-1 at the first evaluation had a normal value at the second. Plasma free thyroxine was decreased in 3 patients, who were therefore given a replacement dose of L-thyroxine.

Clinical follow-up showed a decrease of height SDS for age in the 3 irradiated groups, the mean cumulated change in the 3 years following BMT being -1.4 ± 0.2 SD in group 1, -0.1 ± 0.4 in group 2, and -0.4 ± 0.2 in group 3, while group 4 had a catch-up of 1.5 ± 0.5 SD. The changes were significant in groups 1 and 4 only. Comparing growth of one monozygotic twin of group 1 with that of his brother (donor) showed a difference of 17.5 cm in final height. Among 8 patients with congenital immune deficiency and growth retardation at the time of BMT, those conditioned by chemotherapy alone had significant catch-up growth while those conditioned by X-rays (a single 8 Gy exposure) did not.

The authors conclude that the total radiation dose is critical for growth evolution, as is the fractionation schedule. For the TBI doses and the interval since BMT studied, they did not find correlation between GH or IGF-1 and the height loss. The rapidity of decreased growth velocity after TBI and the comparison between monozygotic twins lead them to suggest that radiation-induced skeletal lesions are partly responsible for the decreased growth.

Brauner R, *et al.* *Arch Dis Child* 1993;68:458-463.

Editor's comment: This protracted study of a large group of children who underwent BMT points out the discrepancy between their growth and the results of repeated measurements of GH secretion or plasma IGF-1, and the clear relationships between transplantation conditioning and growth in the following years. Only the group of children who had been exposed to a single exposure of 10 Gy failed to grow. Those given a single dose of 8 Gy or a fractionated dose of 12 Gy had no serious growth decrease, and those conditioned by chemotherapy alone had catch-up growth. In the group given 10 Gy, there was no good agreement between growth and the results of GH and IGF-1 measurements.

Regarding GH values, the study demonstrated that GH levels are seldom subnormal in children subjected to BMT, and that a second evaluation several years later usually does not evidence delayed changes. However, the data suggest that the protocol of conditioning with 10 Gy in a single dose poses a serious risk of partial GH deficiency and that GH-deficient patients given GH at standard doses normalized their growth velocity, a fact that favors the contribution of hormonal deficiency to the decreased growth of these patients. Conditioning by a fractionated schedule of irradiation or the use of chemotherapy is certainly preferable.

Jean-Claude Job, MD

Growth and Growth Hormone in Children During and After Therapy for Acute Lymphoblastic Leukaemia

Caruso-Nicoletti et al studied growth hormone (GH) secretion prospectively in (1) a group of 50 children with acute lymphoblastic leukemia (ALL) over a 2- to 5-year period following diagnosis and (2) in a group of 12 long-term survivors. Subjects in the longitudinal group had a median age at diagnosis of 5 years and were treated according to protocols of the Italian Group of Paediatric Haematology Oncology with combined chemotherapy as induction and post-remission therapy over 2 years. Children labeled as high or average risk received preventive central nervous system therapy, including intrathecal methotrexate, plus cranial irradiation up to 18 Gy, divided in 10 fractions over a 2-week period. Height was measured at diagnosis, every 3 months during treatment, and every 6 months post-treatment using a Harpenden stadiometer. In patients receiving only chemotherapy (n=8) height was measured yearly. Bone age was determined according to the TW2 method in 31 patients at diagnosis and 19 at the end of therapy. Patients older than 8 years underwent Tanner staging every 6 months. In addition, parents' heights were measured. Arginine-insulin tolerance tests (AITTs) were performed in 19 of the children who underwent cranial irradiation. Ten of these underwent evaluation prior to and immediately after radiation while the remaining 9 underwent a single AITT 2 years after the 2-year cycle of therapy. The group of 12 long-term survivors was evaluated 9.2 ± 2.3 years after diagnosis at a median age of 13.6 years (range, 9.4 to 20.5 years). All long-term survivors received 24 Gy of prophylactic cranial radiations.

All children had a decrease in growth velocity during the first year of therapy. Mean growth velocity standard deviation score (SDS) at 6 months was -2.48 in patients who received radiotherapy (RT) and -1.22 at 1 year. During the second year of treatment, growth velocity returned to near normal, and during the third year children showed a catch-up growth with a mean growth velocity SDS of +1.08 in children receiving RT and +1.19

in the others (NRT). Mean height SDS decreased to +0.58 and +0.04 in RT and NRT patients, respectively, during the fourth and fifth years. Bone age after discontinuation of therapy had increased from that at diagnosis in a linear manner. All patients tested had positive GH responses to at least 1 stimulation test, both at diagnosis and after RT. In addition, all patients tested 3 to 5 years after diagnosis had normal GH responses. In the group of long-term survivors the mean height SDS was $+0.23 \pm 0.83$ at diagnosis and -0.25 ± 0.69 at follow-up. Bone age SDS at follow-up was 0.97 ± 0.2 and pubertal development was normal. Mean age of menarche in the long-term survivors was 10.6 ± 1.5 years.

Caruso-Nicoletti M, Mancuso M, Spadaro G, et al. *Eur J Pediatr* 1993;152:730-733.

Editor's comment: This is an interesting study and its results need to be contrasted with those of Schriock et al reported in GGH (1991;7[4]:15). In that study, a significant mean final height decrement in survivors of ALL who were less than 12 years at diagnosis was observed. Similar findings were recently reported by Katz et al (*J Pediatr* 1993;123:546-552) and attributed to cranial irradiation. The authors of the present study suggest the differences between their results and other studies may be due to the dose of radiation, the interval during which the radiation was administered, or patient age at the time of radiation. It is interesting that they found normal GH secretion in their long-term survivors despite their receiving 24 Gy radiation. The growth deceleration with chemotherapy and radiation followed by increased velocity associated with the cessation of therapy is encouraging. The mean age of menarche is somewhat surprising as it is somewhat lower than might be expected in American girls.

William L. Clarke, MD

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Insulin-Like Growth Factor 2 and Growth

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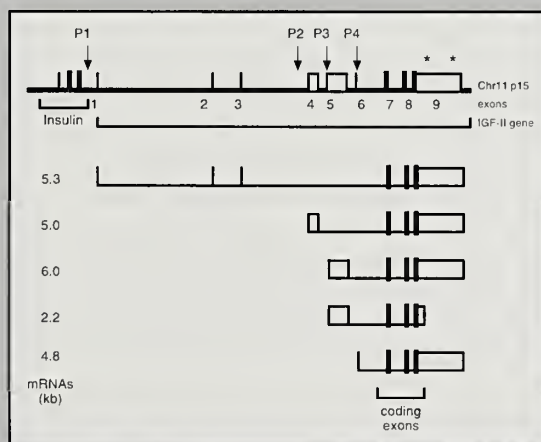
Insulin-like growth factors 1 and 2 (IGF-1 and IGF-2) are polypeptides involved in metabolism, growth, and cell differentiation.¹ IGF-1 is mainly produced during the postnatal growth period. It is involved in statural growth, exerting an endocrine mechanism of action on certain tissues, including cartilage. Furthermore, autocrine/paracrine actions are described in various tissues expressing high levels of IGF-1.¹

In contrast to IGF-1, IGF-2 seems to play a role predominantly during fetal development. However, in human as well as in other mammals, elevated IGF-2 levels persist in serum after birth and even increase during childhood and in young animals. This suggests an endocrine role for IGF-2. IGF-2 is also produced in many tissues, where it has an autocrine/paracrine mechanism of action.¹ The locus of the IGF-2 gene is chromosome 11p15.5, which contains 9 exons. Different IGF-2 mRNAs are expressed, depending on the promoter used among the 4 promoters (P1 through P4) of the IGF-2 gene and on tissue type and stage of development (Figure 1).^{2,3}

IGF-2 AND FETAL GROWTH

IGF-2 plays an important role in fetal growth and differentiation. It is expressed very early in development. In mice, transcripts are detected as early as the blastocyst stage.⁴ IGF-2 expression is found in different types of cells, such as muscle, cartilage, spinal ganglia, hepatocyte, and lung. During fetal life, IGF-2 expression varies relative to the developmental stage. The regulation of IGF-2 expression in the fetus is poorly defined. However, nutrition does not appear to play a central role.⁵ Direct evidence for the physiologic role of IGF-2 in embryonic growth was provided by the work of De Chiara et al.⁶

Figure 1



Structure of the human IGF-2 gene and mRNA species.¹⁻³ Arrows indicate the 4 promoters (P1-P4) alternatively used depending on the tissue and developmental stage concerned. The 5.3 kb mRNA P1 is expressed only in the adult liver. The other mRNA (P2-P4) are expressed in all fetal tissues and in nonhepatic adult tissues.

* Indicates 2 alternative polyadenylated sites.

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Chimeric mice were obtained by gene targeting; IGF-2 gene function was abolished for only 1 allele. The results demonstrated the relation between the loss of function of this 1 allele and a growth-deficiency phenotype, at least at a stage as early as embryonic day 16 and persisting after birth. The size of the heterozygous mice was approximately 60% of the normal size. Moreover, IGF-2 mRNA expression of the intact allele was 10-fold less in heterozygotes than in wild-type embryos. Further studies using these transgenic mice were extended to several generations.⁷ The homozygote mutant mice, in which both alleles were disrupted, did not exhibit any difference from heterozygotes. This indicated no IGF-2 gene-dose-dependent effect on growth. Furthermore, genetic evidence is provided indicating that the IGF-2 locus is subject to parental imprinting: only the paternally derived IGF-2 allele is expressible.⁷

IGF-2, CELLULAR GROWTH, AND DIFFERENTIATION

The IGFs stimulate cell differentiation and DNA synthesis in a large number of cell types, including chondrocytes, fibroblasts, steroidogenic cells, muscle, and lung cells. IGF-1 is more often a better mitogen than IGF-2.¹ Therefore, IGFs have an important role in many organs. For example, IGFs play a major role in muscle differentiation. They have been shown to regulate expression of 3 specific genes: skeletal muscle myogenin, smooth muscle, aortic elastin, and cardiac β -myosin heavy chain genes.⁸ Interestingly, autocrine production of IGF-2 has been detected during skeletal myogenic differentiation.⁸ In lung, they are also involved in organogenesis, compensatory hypertrophy, and repair following injury.⁹ Although IGF-1 and IGF-2 are produced by human lung cells at various stages of development, IGF-2 seems to be more abundant, especially in the fetus.⁹ The development and maintenance of differentiated cell function suggests the role of IGF-1 and IGF-2, particularly in the adrenal gland and in the ovary.¹⁰

IGF-2 AND TUMOR

Increased IGF-2 mRNA expression has been detected in numerous human tumors, including osteosarcomas, nephroblastomas, colon carcinomas, liposarcomas, mammary carcinomas, hepatoblastomas, liver carcinomas, leiomyomas, and leiomyosarcomas.^{1,2,11-14} Autocrine/paracrine IGF-2 production can be involved in both tumorigenesis and tumor growth. Activated P1, usually detected only in adult liver, disappeared in

hepatocarcinoma, while P3 and P4 are activated, as in fetal liver.¹³ IGF-2 DNA demethylation could play a role in IGF-2 gene expression in some tumors such as hepatocarcinoma and leiomyoma.^{13,14}

Chromosomal abnormalities have been reported in some tumors (Wilms' tumors, rhabdomyosarcomas, hepatoblastomas, hepatocellular carcinomas, breast tumors) where loss of an allele was detected in the short arm of chromosome 11.¹⁵ Most chromosomal abnormalities occurred in embryonal tumors, which are often associated with the Beckwith-Wiedemann syndrome.^{13,16-19} These anomalies are found in the 11p13-15 region, suggesting the presence of a tumor suppressor gene. A tumor suppressor gene associated with Wilms' tumor (WT1) was isolated, and mapped to the 11p13 locus.^{20,21} In these tumors, overexpression of IGF-2 was detected; this may have an autocrine effect in tumor progression. Drummond et al²² also have shown by in vitro studies a direct effect of WT1 on the transcription of IGF-2 from the proximal region of P3. These results suggested that functional loss of WT1 may result in increased expression of IGF-2. In cases of sporadic tumors, a preferential 11p15 loss of maternal alleles was detected.²³ In other tumors associated with the Beckwith-Wiedemann syndrome loss of heterozygosity in the 11p15 maternally derived chromosome was found, along with a paternal isodisomy. This suggests the role of parental imprinting and, also, an apparent 2-fold increase in gene dosage of the active IGF-2 allele.^{19,24} A recent report²⁵ demonstrated that the IGF-2 gene was expressed from the paternal allele in human fetal tissues, and expression can occur biallelically in Wilms' tumor. Therefore, a relaxation of imprinting may play a role in cancer development.

IGF-2 IMPRINTING AND METHYLATION*

DNA methylations are heritable, and reversible modifications are implicated in gene control. A link was observed between IGF-2 gene expression and DNA demethylation depending on tissue types.^{13,14}

Genomic imprinting controls the expression of several genes from only 1 allele, either maternally or paternally derived. For example, the IGF-2 gene is paternally expressed⁷ and the IGF-2 receptor is maternally expressed.²⁶ Regulation of imprinting is still an unknown phenomenon. However, DNA methylation was shown to be variable for maternally versus paternally derived chromosomes.²⁷

As mentioned above, it was reported that in mice, as in humans, the IGF-2 gene was subject to a

*See Genetics Glossary insert in *GGH* Vol. 9, No. 1.

specific parental imprinting.^{7,25} A parental methylation difference was detected for the mouse IGF-2 gene, suggesting that the paternal chromosome, which had the active allele, was more extensively methylated.²⁸ Moreover, only specific DNA sites located in the coding region, but not in the promoter region, were determined to be the target of such methylation modification.

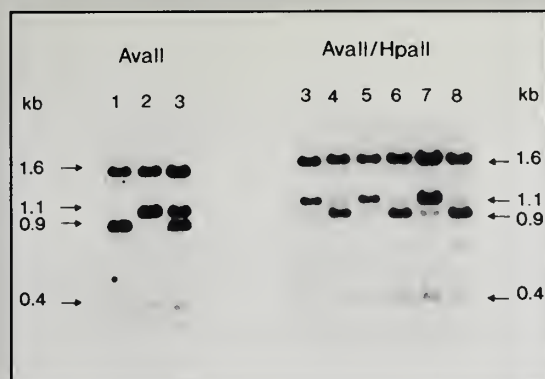
In order to determine whether DNA methylation was linked to differential imprinting, Southern blot analyses were done using leukocyte DNA from unrelated controls. We determined that only 1 allele was always demethylated on the IGF-2 gene (Figure 2).¹⁹ We located this particular phenomenon in exon 9 of the IGF-2 gene. This was extended to familial analysis, and we observed that the maternally derived allele was always demethylated, whereas the paternally derived allele was always methylated. This was an IGF-2-specific phenomenon that was not detected for insulin or calcitonin genes, which also are located on the short arm of chromosome 11. Thus, it appeared that the IGF-2 gene was subjected to a parental allele-specific methylation.¹⁹ Although no direct evidence for a link between imprinting and DNA methylation has been provided, an increasing number of studies support the fact that DNA methylation may be the controlling factor for imprinting.²⁹

GENOMIC IMPRINTING IN HUMAN DISEASE AND IGF-2

In specific syndromes such as the Angelman syndrome and Prader-Willi syndrome, the same 15q11-13 locus has been implicated. This is a typical example of pathology in which allele-specific imprinting leads to different anomalies. De novo deletions occur in both cases for the same locus, but exclusively on the paternal chromosome in the Prader-Willi syndrome and exclusively on the maternal chromosome in the Angelman syndrome.³⁰

It is likely that abnormalities in IGF-2 imprinting also are present in pathologic processes. The observation that IGF-2 imprinting is altered in some Wilms' tumors²⁵ suggests that this event also may occur in the Beckwith-Wiedemann syndrome. Moreover, family studies have indicated that there is a link between Beckwith-Wiedemann syndrome and the chromosome 11p15 region where the IGF-2 gene is localized.³¹ Both loss of maternal 11p15 heterozygosity and paternal isodisomy have been described in Beckwith-Wiedemann syndrome patients.^{13,19,24,32} We analyzed genomic DNA in 29 Beckwith-Wiedemann syndrome patients. Loss of heterozygosity was detected in 5 of the 22

Figure 2



Southern blot analysis of control leukocyte DNA, digested with *Ava* II or *Ava* II/*Hpa* II, using IGF-2 cDNA as probe. *Ava* II revealed the polymorphic alleles (1.1 kb and 0.9 kb). *Hpa* II cleaved only the demethylated allele.¹⁹

1. homozygote for the 0.9-kb allele
2. homozygote for the 1.1-kb allele
- 3-8. heterozygotes for the 0.9- to 1.1-kb alleles

informative cases, the maternally inherited allele was always the lost allele.¹⁹ In most of the cases, mosaicism of cells was observed, with both normal cells and cells with loss of heterozygosity present. This supports the concept of a postzygotic event. Loss of heterozygosity was detected in tumor tissues and in nontumor tissues such as leukocytes and lingual tissue obtained after partial glossectomy because of invalidant macroglossia. This revealed that loss of heterozygosity is not a unique element needed for tumorigenesis. However, detection of loss of heterozygosity in leukocytes could be a useful tumor prognostic parameter for regular follow-up of these Beckwith-Wiedemann syndrome patients. Effectively, among the 5 cases where loss of heterozygosity was observed, 4 had a tumor.¹⁹ For the 29 Beckwith-Wiedemann syndrome patients, we also analyzed the IGF-2 parental allelic methylation. Abnormal results were found only in pathologic tissues, not in leukocyte DNA.¹⁹ Therefore, a relationship between IGF-2 gene expression, methylation, imprinting, and loss of heterozygosity seems to exist in the pathology of the Beckwith-Wiedemann syndrome, but the sequence of events remains to be established.

In the future, other pathologic conditions in which IGF-2 could be implicated, such as intrauterine growth retardation, should be examined in regard to these new findings and concepts.

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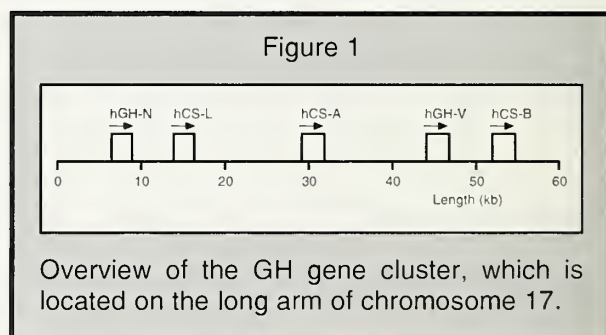
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Placental Growth Hormone Variant: A Specific Marker of Pregnancy With Still Unknown Functions

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The human placenta has recently been shown to express the GH-V gene specifically, leading to the production of placental growth hormone (placental GH). The GH-V gene belongs to a family of 5 GH/chorionic somatomammotropin (CS) genes located in a 58-kbp cluster on the long arm of chromosome 17. These 5 GH/CS genes are aligned in the same transcriptional orientation (GH-N, CS-L, CS-A, GH-V, and CS-B from 5' to 3'). They show a high degree of sequence identity (91% to 99%) and have the same structure (4 introns/5 exons) (Figure 1). GH-N gene expression in the pituitary yields human GH. The CS-A and CS-B genes encode for the same protein known as placental lactogen (PL) or CS. PL is secreted at very high levels during pregnancy, with maternal serum concentrations of up to 10 μ g/mL at term. Although the CS-L gene was presumed to be nonfunctional, recent evidence for its expression in the placenta, as well as in the pituitary, have been reported.¹ Two different size transcripts are generated from the GH-V gene: a major GH-V mRNA translated into the 22-kd placental GH²; and a minor one, named GH-V2 mRNA, resulting from an alternate splicing with the retention of intron 4 in the mature mRNA. The translated protein has neither been defined nor shown to be secreted. The carboxyl-terminal configuration of the GH-V2 protein is consistent with a membrane spanning region, suggesting the possibility that GH-V2 may be an integral membrane protein.³



The major GH-V mRNA is translated into a mature secreted protein: the placental GH. The amino acid sequence of placental GH differs from that of the GH-N protein by 13 residues. Placental GH is more basic than pituitary GH and contains a unique N-linked glycosylation site at asparagine 140. It is produced as 2 different size variants corresponding to a glycosylated 25-kd form and a nonglycosylated 22-kd form.⁴⁻⁶ Placental GH is produced by the syncytiotrophoblast in vivo and in vitro.⁷⁻⁹

ASSAYS FOR PLACENTAL GH

Placental GH can be detected in the maternal blood and is distinguishable from pituitary GH on the basis of its reactivity with 2 monoclonal antibodies (MAbs: K24 and 5B4) raised against purified pituitary GH.⁴ The 5B4 MAb reacts with the N-terminal epitope of both pituitary GH and placental GH. The K24 MAb reacts with an internal epitope and recognizes pituitary GH exclusively. Therefore the difference between the results obtained with the 2 assays provides an estimation of the concentration of placental GH

present in the serum. Cross-reactivity with CS is $<0.005\%$ in both systems. Recently, the laboratory of G. Hennen (Liège, Belgium) has produced a monoclonal antibody (MAb E8) against purified placental GH from *Escherichia coli*. It recognizes an internal epitope of the molecule and is strictly specific for placental GH. A very good correlation ($r=0.93$) between the levels of placental GH measured with the 5B4 radioimmunoassay and the E8 radioimmunoassay is observed in the maternal plasma near term (38 weeks of amenorrhea).

PLACENTAL GH LEVELS IN MATERNAL SERUM DURING NORMAL PREGNANCY

Studies of GH physiology in pregnant women have revealed that in the early stages of pregnancy (up to 15 to 20 weeks), pituitary GH is present in significant amounts in the maternal circulation, displaying a highly pulsatile 24-hour serum concentration profile. Later on, from 15 to 20 weeks up to term, increasing concentrations of placental GH replace pituitary GH, the levels of which decrease progressively to the point of undetectability (Figure 2).^{4,10} Placental GH is not detected in the fetus or in the cord blood of the newborn.⁴ In contrast to pituitary GH, placental GH is secreted in maternal serum in a nonpulsatile manner and declines rapidly following delivery, as expected for a placental hormone.¹¹ There is a drastic fall in placental GH at the onset of labor, probably due to the decrease in uteroplacental blood flow and the release of placental proteases.

PLACENTAL GH IN MATERNAL SERUM DURING ABNORMAL PREGNANCIES

Recently, we have measured placental GH levels in normal pregnancy and in pregnancies complicated by intrauterine growth retardation.¹⁰ Interestingly, maternal plasma samples obtained after 31 weeks of amenorrhea until the initiation of labor in cases of intrauterine growth retardation contained significantly ($P<0.001$) low levels of placental GH. Plasma insulin-like growth factor 1 (IGF-1) levels also were lower than normal ($156.0 \pm 25.5 \mu\text{g/L}$ vs $285.1 \pm 40.8 \mu\text{g/L}$). These results suggest a relationship between placental GH levels in the maternal plasma and the development of the fetoplacental unit. In contrast, the levels of placental GH were within the normal range in the sera of pregnant women with anencephalic fetuses.

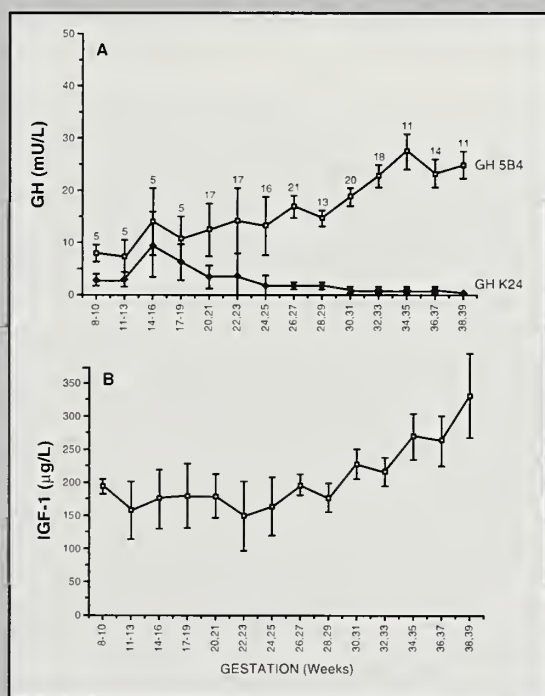
PHYSIOLOGIC ROLE OF PLACENTAL GH

The precise functions of placental GH during pregnancy still are poorly known. A physiologic role for a placental GH variant is suggested since it binds to a

GH-binding protein and to GH receptors from pregnant rabbit liver cells, as well as to human placental tissues.^{12,13} Placental GH may be biologically active mainly as a somatogen and less as a lactogen,¹⁴ since its somatotrophic activity on human GH-V has been illustrated by a 40% to 90% increase in size of transgenic mice bearing the human GH-V gene, as compared with controls.

However, direct action of placental GH on fetal growth seems very unlikely because placental GH is not detected in the fetal circulation. A metabolic role in the mother is suggested by the positive correlation observed between placental GH levels and IGF-1 levels in maternal blood in late pregnancy, when pituitary GH is no longer secreted.¹⁵ In addition, maternal IGF-1 levels do not seem to be

Figure 2



Transverse study: maternal plasma GH (A) and IGF-1 (B) levels during pregnancy ($n=186$).¹⁰ Results with GH 5B4 reflect the concentrations of placental GH, while results with GH K24 reflect pituitary GH. Each point represents the mean \pm SEM of values from individual samples obtained in pregnant women at the indicated periods of pregnancy, expressed as weeks of amenorrhea. Each period consists of 3 weeks until the 20th week and of 2 weeks thereafter. The number of individual assays for GH and IGF-1 for each gestational stage is indicated in panel A of the figure on top of the vertical bars.

solely under the control of pituitary GH during pregnancy, as shown by studies of acromegalic women in whom, despite the apparent stability of pituitary GH levels, serum IGF-1 levels increase during pregnancy.¹⁶ Through its somatogenic activity, placental GH may be involved in maintaining high levels of energy-yielding nutrients in the mother-to-be transferred into the fetoplacental unit. Indeed, during the last trimester of pregnancy, fetal growth is normally constrained by maternal factors. A direct role of placental GH on placental development and on its multiple endocrine and immunologic functions (and, therefore, indirectly on fetal growth) is suggested by the fact that placental GH is subject to autocrine control within the syncytiotrophoblast, which produces placental GH and expresses GH receptors.^{7-9,13}

In conclusion, the further development of assays for detecting placental GH will be of help in elucidating the physiologic role of this hormone, which is specific for pregnancy. The lower levels of placental

GH observed in most cases of intrauterine growth retardation suggest that placental GH levels reflect placental biologic activity and may be useful in assessing chronic fetal distress resulting from abnormal placental development and function.

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The Diagnosis and Management of Craniopharyngioma

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The profound effects upon growth and development produced by craniopharyngiomas make these lesions among the most dramatic and challenging abnormalities encountered in medical practice. As early as 1900, Babinski¹ described a patient with sexual infantilism and dystrophic obesity who had a cystic sellar-suprasellar lesion, almost certainly a craniopharyngioma.

ETIOLOGY AND PATHOLOGIC ANATOMY

Craniopharyngiomas are generally believed to be developmental lesions, believed to arise from remnants of Rathke's pouch. These embryonic remnants occur as epithelial rests, deposited between the tuber cinereum and the pituitary gland itself, along the tract of an incompletely involuted hypophyseal-pharyngeal duct; craniopharyngiomas can arise anywhere along this pathway. It has also been postulated that craniopharyngiomas might arise from squamous metaplasia of normal cells of the pars

intermedia situated along the pituitary stalk.² From the standpoint of size, location, contents, pathologic appearance, and overall clinical behavior, craniopharyngiomas encompass a broad biologic spectrum. At the one extreme are minute tumors of microscopic proportions situated wholly within a normal pituitary gland. At the other and more common extreme, there are larger tumors whose progressive growth enables them to compress the pituitary gland and stalk, optic apparatus, and hypothalamic structures. These larger lesions may extend into the third ventricle, causing hydrocephalus. Craniopharyngiomas can be solid or cystic, and the overwhelming majority exhibit both features. The cyst contents, although classically described as "machinery oil" in appearance and consistency, can range from a shimmering cholesterol-laden fluid to a brown-black purulent sludge admixed with desquamated debris. Calcification is a common feature of craniopharyngiomas, ranging from microscopic specks to palpable and even bone-like concretions of considerable size. Pathologically, the epithelial elements comprising these tumors range from cuboidal to columnar to squamous in appearance.

Topologically, approximately 60% to 80% of craniopharyngiomas arise in the suprasellar region.³ Approximately 30% to 40% of craniopharyngiomas originate within the sella, resulting in its enlargement in a fashion similar to that seen with pituitary

Table 1
Symptoms and Signs of 82 Pediatric Patients With Craniopharyngiomas

Symptoms	Incidence (%)	Signs	Incidence (%)
Headache	71	Papilledema	35
Visual loss	55	Visual field deficit	52
Endocrine deficiency	45	Somatic retardation	45
Polydipsia/polyuria	21	Sexual retardation	44
Personality and mental changes	12	Diabetes insipidus	21

adenomas.⁴ Rare examples of craniopharyngiomas wholly situated within the third ventricle, optic chiasm, or sphenoid bone have been reported.

EPIDEMIOLOGY

In the United States, craniopharyngiomas represent approximately 3% of intracranial tumors. They are more frequent in children than adults, comprising approximately 9% of childhood brain tumors. There is an incidence peak in childhood (ages 5 to 10 years), and then the frequency for the 3rd to 7th decades is relatively constant; however, there is a tendency towards a second smaller peak at 50 to 60 years of age. Sex distribution is nearly equal, with a slight male predominance.

CLINICAL PRESENTATION

The clinical presentation of craniopharyngiomas is determined by the age of the patient and the size and location of the tumor.⁵⁻⁷ In general, symptoms can be categorized as endocrine, visual, cognitive, and those deriving from increased intracranial pressure (Table 1).

Virtually all children with craniopharyngiomas will have an abnormal growth curve and an absence or delay in development of secondary sexual characteristics. In young adults, endocrine symptoms are often subtle, particularly those related to partial

hypopituitarism. The effects of moderate hyperprolactinemia from infundibular stalk or hypothalamic compression are generally more obvious, especially in young women, in whom prolactin (PRL) elevations manifest as amenorrhea and galactorrhea. Diabetes insipidus may occur as part of the presenting symptom complex in both children and young adults.

ENDOCRINE DIAGNOSIS

The endocrine diagnosis of craniopharyngioma rests on physical signs and laboratory studies (Table 2). Laboratory measurements include basal and provoked tests of pituitary-hypothalamic function as clinically indicated. These include basal determinations of growth hormone (GH), insulin-like growth factor type 1 (IGF-1), PRL, cortisol, thyroid function (thyrotropin and thyroxine [T_4]), gonadotropes (follicle-stimulating hormone [FSH], luteinizing hormone [LH]), testosterone, and estradiol. Determination of alpha subunit levels and corticotropin can occasionally be helpful. The major GH-dependent IGF-binding protein, IGFBP-3, can also be measured.

An insulin tolerance test with measurement of cortisol and GH offers a helpful dynamic evaluation of the pituitary-hypothalamic axis. If diabetes insipidus is suspected, urine and serum osmolality determinations or a water-deprivation test may be helpful.

Table 2
Deficiencies in Endocrine Testing: 82 Pediatric Patients Presenting With Craniopharyngiomas

Hormone Deficiency	Patients No. Abnormal/No. Tested	Incidence
Growth hormone	10/40	25%
Corticotropin	30/71	42%
Thyrotropin	19/69	28%
Gonadotropin	23/49	47%
Vasopressin	10/45	21%
Patients with at least 1 hormone deficiency	40/77	52%

IMAGING DIAGNOSIS

Magnetic resonance imaging (MRI) is the diagnostic procedure of choice for craniopharyngioma (Figures 1A and 1B). Both solid and cystic components are identified, along with important anatomic relationships and various extensions of tumor and cyst outside of the immediate suprasellar region. With high-resolution MRI, cerebral angiography is generally reserved for cases of presumed vascular tumors or those that have a particularly difficult relationship to blood vessels in the region. Computed tomography (CT) still plays some role in the anatomic diagnosis of craniopharyngiomas and has the advantage of showing calcifications and some aspects of bony distortion associated with the tumor more effectively than does MRI.

MANAGEMENT

In newly diagnosed patients, maximum safe tumor resection is often a reasonable initial goal. In many instances, this can be achieved via craniotomy,^{8,9} although in selected cases transsphenoidal resection provides safer access.^{4,10} In some cases, complete excision will require a combination of both approaches. Tumors not associated with sellar enlargement generally arise and remain suprasellar, and therefore are best managed by a transcranial (pterional or subfrontal) route. During the course of tumor resection it will usually become evident whether complete removal is a safe and feasible strategy.¹¹ Aggressive attempts to remove tumor fragments that are tenaciously adherent to neural and vascular structures will be accompanied by an unacceptable functional cost.^{12,13} Other tumors will be less adherent and complete excision can be safely achieved.

As a rule, craniopharyngiomas associated with sellar enlargement can be regarded as subdiaphragmatic in origin. Even though such tumors may exhibit significant intracranial extension, they invariably maintain an extrapial and extra-arachnoid disposition. Accordingly, they remain amenable to complete excision via a transsphenoidal route.

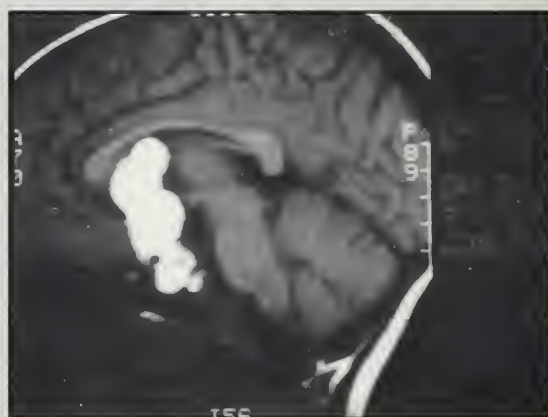
Completeness of surgical removal can usually be confirmed by postoperative imaging studies. When complete removal is not feasible, some form of postoperative radiation therapy is generally recommended, except in very young children.¹⁴

The management of recurrent craniopharyngiomas is considerably more complex, for therapeutic goals must be especially well defined. In some cases – and despite the technical demands of reoperation – total resection can still be achieved. For many recurrent tumors, however, palliative surgery is often the most realistic goal. Recurrent

Figure 1A
**Sagittal View of a Craniopharyngioma
With Retrosellar Extension**



Figure 1B
**Sagittal MRI View of a Craniopharyngioma
With a Complex Suprasellar
Extension Into the Third Ventricle**



lesions with a significant cystic component can often be treated by repetitive aspiration. This can be achieved by inserting a silastic tube attached to an Ommaya reservoir into the cyst cavity. Alternatively, the transsphenoidal insertion of a silastic tube from the tumor cavity into the posterior nasal space can provide prolonged drainage, or bleomycin sulfate may be instilled to shrink and toughen the cyst wall.

Stereotactic needle aspiration of fluid contents may be remarkably effective in reversing symptoms and signs. In some cases a radioactive isotope (colloidal ³²P, yttrium, or gold) may be instilled into the cyst cavity to provide local beta particle-mediated radiotherapy. Radiosurgery, delivered by gamma knife or by stereotactic linear accelerator, may also provide dramatic results in patients who are not suitable surgical candidates.

RESULTS OF SURGERY

This analysis will focus on a series of pediatric patients with craniopharyngiomas treated at the Mayo Clinic in Rochester, Minnesota and followed for at least 5 years.

The study group consisted of 82 children operated upon between 1950 and 1983. The age range was from 2 to 20 years, with a mean age of 12 years. Fifteen of the children were younger than 8 years of age at the time of surgery. All had developed symptoms and signs before age 16. The children were followed for a mean of 9.7 years.

The symptoms, signs, and endocrine deficiencies of the entire group at the time of surgery are presented in Tables 1 and 2 (page 7). Data on follow-up status are presented in Figure 2 and percent survival in Figure 3.

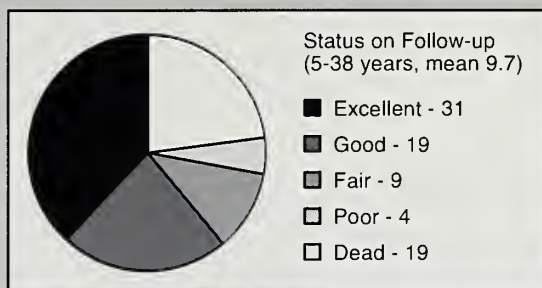
Management of the 82 children was categorized as follows:

- Total surgical resection only was performed on 31; total surgical resection followed by radiotherapy was performed on 6; subtotal resection only on 17; subtotal resection followed by radiotherapy on 17; minimal surgery (biopsy or cerebrospinal fluid shunt) on 8, and minimal surgery followed by radiation therapy on 3.
- Of the 37 patients with total surgical resection, 6 had postoperative radiotherapy. Satisfactory outcome, defined as long-term survival with good or excellent quality of life, was achieved in 78% (29 children). There were 2 operative deaths, and 6 recurrences (16%) in spite of total resection. The 5- and 10-year survival rates were identical at 90%. On long-term follow-up, 4 of the 37 children (11%) had died.
- Of the 34 patients with subtotal surgical resection, 17 had postoperative radiation therapy. A satisfactory outcome, as defined above, was achieved in 35% (12 children). Recurrent disease developed in 17 (50%). There was 1 operative death. The 5-year survival rate was 75%; the 10-year survival rate fell to 65%. On long-term follow-up, 14 of the 34 children (41%) had died.
- Radiotherapy consisted of conventional teletherapy to a mean dose of 4,450 Gy, usually in 180-Gy daily fractions. The dose range was 2,650 to 6,300 Gy. These data support a policy of maximum safe tumor resection as the initial recommendation for management of children with craniopharyngioma.

CONTROVERSIES IN MANAGEMENT

Various forms of subtotal removal of craniopharyngiomas or their contents, followed by radiation therapy, have been advocated.¹⁵ Although radiation therapy is of proven benefit in delaying recurrence and controlling tumor growth, most forms of radiation

Figure 2
Results of Management of
82 Craniopharyngioma Patients



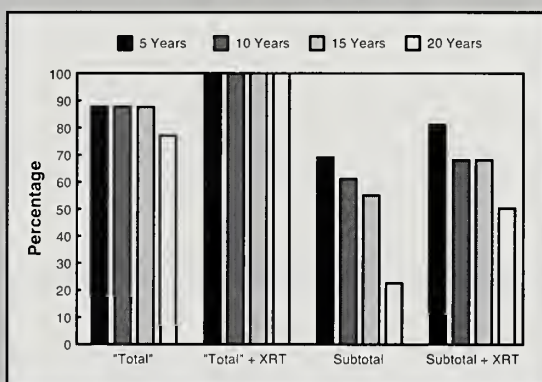
Excellent = independent, no neurologic deficit

Good = independent, stable neurologic or visual deficit

Fair = dependent but functional, with neurologic or visual deficit

Poor = dependent, nonfunctional

Figure 3
Overall Survival Statistics in
71 Pediatric Patients



Total surgical resection	= 31 patients	} 37 patients
Total surgical resection and radiotherapy	= 6 patients	
Subtotal removal	= 17 patients	} 34 patients
Subtotal surgical resection and radiotherapy	= 17 patients	

29 of the 37 total surgical resection patients (78%) had satisfactory outcome (defined as long-term survival with good or excellent quality of life) versus 12 of the 34 subtotal surgical resection patients (35%)

therapy currently given for craniopharyngioma produce hypothalamic and pituitary damage.^{16,17} There is also a small but constant risk of radiation-induced optic neuropathy leading to blindness; diffuse brain damage leading to dementia; focal brain necrosis affecting the hypothalamus or septal region; vascular pathology leading to occlusion and a moyamoya type vasculopathy; and the late induction of secondary tumors, many of which are malignant. New forms of radiation therapy are being evaluated, and should represent a major advance in the avoidance of such complications. It is also hoped that they will be more effective in destroying the tumor and in reducing its potential for recurrence.

One must recognize the selection bias that affects previously reported retrospective studies. Rapidly growing large tumors that cause progressive visual loss and hydrocephalus demand surgical management, and the outcome of many of those patients reflects the aggressiveness of the tumor.¹⁸ Those patients with small, indolent, relatively asymptomatic tumors are overrepresented in the group of patients treated by less radical surgery and radiation. Naturally, the end results reflect the less aggressive nature of their tumors.

Because each craniopharyngioma is different, it is important to individualize the plan of management for each patient, taking into account what is known about the anatomy and biology of the tumor. In previously untreated patients, several issues should be addressed. Total removal remains a reasonable goal for many tumors, especially those with enlargement of the sella and no major involvement of or attachment to the hypothalamus or the optic apparatus. In infants and children who are growing

normally, it is occasionally prudent to delay therapy until growth is complete.

CONCLUSION

The goal of treatment – a neurologically intact patient living as normal a life as possible – is accomplished by using a judicious combination of careful surgery, meticulous medical and endocrine management, and appropriate radiation therapy. Improvements in diagnosis and in the technical and conceptual aspects of medical, surgical, and radiotherapeutic management should lead to continuing improvement in the prognosis for patients with craniopharyngiomas.

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Abstracts From the Literature

Transsphenoidal Surgery for Pituitary Adenomas in Children

The authors report the surgical results of transsphenoidal surgery for pituitary adenomas done on 66 patients; all were under 16 years of age at the time of surgery. The sex incidence was equal. Ninety-four percent (62 of 66) clinically showed evidence of hormonal hypersecretion; 36 secreted excessive ACTH producing Cushing's syndrome; 18 patients had hyperprolactinemia; 8 patients had gigantism.

ACTH producing adenomas (36) accounted for 55% of the cases. Thirty-three of these had normal sellar X-rays and the other 3 had Nelson's syndrome. In addition, neither CT or MRI scans proved very accurate in localizing this type of adenoma as these scans proved helpful in only 7 of 24 cases, in which at least 1 of the 2 types of scans was done. Eighteen of 26 with selective removal of the tumors and 5 of 8 requiring subtotal hypophysectomy were cured (70%). Ten failures occurred initially, but 5 of 6 with repeat subsequent surgery were successful. The overall surgical success rate was 78% (28 of 36), although 5 of the 28 required 2 operations. Temporary diabetes insipidus (12 cases)

was common, but permanent diabetes insipidus was uncommon (1 case). In this series, ACTH microadenomas occurred within the confines of a normal sella in 86% (31 of 36) of cases.

Prolactinomas occurred in 7 boys and 11 girls; only 3 girls had galactorrhea. Pubertal delay was common in both sexes. Fifteen of 18 patients with prolactin (PRL) adenomas had some evidence of sellar enlargement by X-ray or tomography. Thirteen of these had suprasellar extension. Parlodel® (bromocryptine mesylate) was often but not uniformly helpful preoperatively or postoperatively. The 4 patients in whom Parlodel was minimally effective, or in whom obvious invasion was present, received radiotherapy. Prolactinomas in boys were particularly difficult management problems, with 6 of 7 showing suprasellar extension at admission and 5 of 7 requiring Parlodel and/or radiotherapy postoperatively. Overall, only 1 (11%) of 9 patients with significant pubertal delay at admission subsequently had adolescent development. Patients with high PRL levels (>500 ng/mL) did poorly in contrast to those with values between 200 to 500 ng/mL.

The 8 children with growth hormone (GH)-secreting adenomas did dismally in contrast to the usual 60% to 80% cure rates in adults. Only 1 patient fared well. Five of the 8 had suprasellar extension. Six of the 8 had enlarged sellas.

Nonsecreting adenomas occurred in 4 patients, 2 of whom had suprasellar extension with visual field changes. The lesions in the other 2 were localized. There was no operative morbidity or mortality in this group.

Twelve (18%) of the 66 required drilling of an incompletely pneumatized sphenoid sinus to reach the sella, which did not in any case limit the surgical procedure. The authors state that second operations by the transsphenoidal route can be done in children with results approaching those seen in adults for the debulking or removal of recurrent or residual lesions. For those lesions not controlled by surgery, the authors recommend medical intervention, not radiotherapy.

Dyer EH, et al. *Neurosurgery* 1994;34:207-212.

Editor's comment: The data in this paper cover the period 1966 to 1992 and were reported from the Department of Neurosurgery at the Centre Medico-Chirurgical Foch in Suresnes, France. The authors document that transsphenoidal surgery is viable in the pediatric age group. Visual scans are helpful in

most instances of non-ACTH-producing adenomas, but of limited value in ACTH-producing adenomas. Unfortunately, the latter make up the majority of adenomas observed in childhood. Incidentally, we are indebted to Dr. Laws, who wrote the article regarding the treatment of craniopharyngioma in this issue, for bringing this article to our attention.

Robert M. Blizzard, MD

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Third International Symposium on Insulin-Like Growth Factors

February 6 - 10, 1994; Sydney, Australia

A Meeting Review by Paul Saenger, MD, Professor of Pediatrics, Department of Pediatrics, Montefiore Medical Center, Bronx, New York

The 3rd International Symposium on Insulin-Like Growth Factors was held in Sydney, Australia, February 6-10, 1994. Approximately 800 clinicians and investigators presented research data in the burgeoning field of insulin-like growth factor 1 (IGF-1) function and action.

Highlights of the talks focused on new data on IGF-1 function in normal embryonic development and the role of IGF-1 in the treatment of diseases of the central nervous system (CNS). Dr. Peter Gluckman, Auckland, New Zealand, and his group presented data suggesting that the paracrine IGF-1 system responds to CNS organ injury with changes in the expression of insulin-like growth factors and their respective binding proteins. In this model using 21-day-old rats, neuronal death is a mixture of necrosis and apoptosis (cell death) and is evident between 12 and 72 hours after CNS injury. IGF-1 mRNA is markedly induced within 24 hours. In contrast, IGF-2 expression is not seen for 7 to 10 days. In adult rats subjected to a hypoxemic-ischemic CNS injury, IGF-1 given intraventricularly caused a

dose-dependent reduction in infarction and neuronal death in all areas studied. The effect appears to be mediated by the IGF-1 receptor. IGF-1 was effective only if given within 2 hours after injury and not if given prior to the injury, suggesting that it acts on mechanisms activated by the hypoxemia-ischemia.

Dr. Gluckman suggested that IGF-1 may act as a survival factor blocking apoptosis. The action of IGF-1 was further determined by local production of IGF-binding proteins (IGFBPs). The neuroprotective action of IGF-1 was blocked by the coadministration of IGF-2. Both are probably competing for the same receptor. In a fetal sheep ischemic injury model, intracerebroventricular IGF-1 was also neuroprotective at very low doses. The data suggest that in the brain IGF-1 acts as a survival factor for asphyxiated neurons and that the effect is dependent on the presence of IGFBP-2 and/or IGFBP-3. This is an endogenous protective mechanism clearly warranting further study as the clinical therapeutic potential is obvious.

Dr. Ron Rosenfeld, Portland, Oregon, reported the results of an IGF-1 treatment trial in patients with primary growth hormone insensitivity from Ecuador. This was the first double-blind, placebo-controlled trial with a cross-over at 6 months. The growth velocity increased from 3 cm/y to 8.5 cm/y at an IGF-1

dose of 120 µg/kg bid. There was little change in the serum levels of IGFBP-3 or the distribution of IGF peptides among IGFBPs while patients were on treatment. There also was little change in the pharmacokinetics of IGF-1 therapy during treatment. The measured increase in growth velocity is significant, but does not match the response in a naive growth hormone-deficient patient treated with daily growth hormone injections. Dr. Rosenfeld concluded that the optimal dose of IGF-1 is still elusive. A different injection schedule or administration of IGF-1 complexed with binding proteins are among the approaches to be explored in the future. Most importantly, the studies of Gluckman and Rosenfeld demonstrate the ability of IGF-1 peptides to act as classical endocrine hormones in clinical trials.

Insulin-like growth factors involve many aspects of growth and development. The study of IGF-1 action has also been carried out by administering IGF to animals by injection or mini-pumps. Transgenic technology addresses the question of overproduction or underproduction with a recently developed embryonal stem cell technology. Here, gene expression for specific hormones can be removed from animals and then the effects can be studied. Dr. Lyn Powell-Braxton, South San Francisco, described her exciting research data with mice lacking a functional IGF-1 gene. These mice are profoundly IGF-1 deficient. This enables investigators to study strains of mice deficient in IGF-1 ("knock-out" mice) from

conception on. Mice with 1 functional IGF-1 allele are 10% to 20% smaller than their normal littermates, and they have lower IGF-1 levels. Mice totally lacking a functional IGF-1 gene progress normally through prenatal development, but more than 95% die at birth. These IGF-1 knock-out mice are just over half the size of their normal littermates. They have severe muscle dystrophy affecting both cardiac and skeletal muscle. The mice have poorly developed diaphragms and lungs, and are unable to breathe. Surviving animals show reduced myelination in nervous tissue. Dr. Powell-Braxton concluded that IGF-1 knock-out mice show severe effects on the development of both the central and peripheral nervous systems and, additionally, produce pronounced dwarfism, muscle underdevelopment, and reduced longevity. Lung development also may be affected, as the lung is one of the organs with the most striking disproportional growth retardation.

The availability of these mouse strains illustrates the importance of the IGF-1 system not only in postnatal but also in embryonic growth. The availability of mouse strains with genetically defined lesions is an increasingly powerful tool in the study of the role of growth factors and hormones throughout development. The availability of these knock-out mice with defined deficits in IGF-1 gene expression permits investigators to dissect the role of IGF-1 not only in development but also in disease pathogenesis.

The First International Meeting of the Growth Hormone Research Society

June 1 - 4, 1994; Aarhus, Denmark

A Meeting Review by Paul Saenger, MD, Professor of Pediatrics, Department of Pediatrics, Montefiore Medical Center, Bronx, New York

Over 400 delegates attended this meeting, which was devoted to clinical and basic science aspects of growth hormone (GH) research. At the meeting, which had attracted a large number of adult endocrinologists, the importance of GH in adults with GH deficiency was examined. Particular emphasis was placed on further study of the regulation of GH secretion, diagnosis, and characteristics of adult GH deficiency, and the effects of GH replacement therapy in adults.

Professor Iain Robinson, London, England, spoke about peptidal and nonpeptidal GH-releasing substances and their interaction with the GH-releasing factor (GRF) neuron. The GRF neuron has to be

viewed as a clearinghouse for a wide variety of afferent neuronal information. He stressed that, in his view, the GH-releasing action of GRF is not its primary function.

The pulsatile GH release is most likely regulated by somatostatin. Somatostatin optimizes the pulse frequency pattern, thus stimulating growth by setting a pulse pattern that is most economical for achieving the maximum growth of peripheral tissues. He could show elegantly that 9 GH pulses per day give more bone growth in the rat than 1 large pulse of GH with a similar area under the curve. The GRF/somatostatin interplay has been studied not only in physiologic settings but also in disease, such as in patients with ectopic GRF production where, even under constant GRF exposure, GH release remains pulsatile.

Professor Robinson reported preliminary data on

12-hour GH sampling in premature infants. In collaboration with Dr. David Dunger, Oxford, England, he was able to show that in 34-week gestation premature infants, GH is already secreted in bursts. The striking difference compared with older children was that their GH level never declined to zero. Peaks of GH were superimposed over a baseline level of 5 to 10 ng/mL of GH. Dr. Robinson indicated that the high GH levels in premature infants suggest that GH probably has important metabolic functions in utero.

Professor Robinson stressed it is far from true that all GRF pulses are associated with a subsequent GH release. It is only due to pulsatile somatostatin that we achieve a GH release after GRF at all.

The major function of GRF, according to Professor Robinson, is to build up GH stores in the pituitary. Indeed, it has been shown that GRF does induce increased GH gene transcription. He cited the *little* mouse, which does not respond to GRF, as an intriguing animal model to study the physiology of GRF action. A single point mutation in the extracellular domain of the GRF receptor renders the *little* mouse resistant to GRF. This then leads to a total failure of postnatal GH cell proliferation in the pituitary and the pituitary GH cell population is near zero. GRF, therefore, exerts a trophic function for growth hormone secreting cells as well.

Professor Robinson stressed further that the pituitary has to be viewed as a plastic organ that can change the number of GH-producing cells in response to afferent input, GRF being among them. Physiologic GRF production is then enmeshed in a feedback loop where GH release exercises a negative feedback. Furthermore, central GH receptors are equally responsive to circulating peripheral GH. There are several afferent inputs for the GRF neuron. These inputs come from GH itself, somatostatin, synthetic GH-releasing peptides (GHRPs), neurotransmitters, and possibly also IGF-1.

Professor Robinson concluded his talk by reviewing the current knowledge of GHRPs. Simply just the fact that GHRPs have an effect in man suggests that there may be endogenous, still elusive, GHRPs produced in the brain. Since synthetic GHRPs are effective, one has to postulate that there are receptors in the brain for these synthetic GHRPs. Whether they are identical to GHRP receptors for endogenous GHRP is not clear. GHRPs work through their own receptors, not through GRF receptors. Furthermore, GHRPs also have a hypothalamic target in addition to a pituitary target. GHRPs act in synergism with GRF and regularize the response to GRF. Elegant studies utilizing anti-GRF show that GRF-Abs interfere with GHRP action. A functioning hypothalamus is required for full GHRP action. Additional effects of GHRP may also influence the firing rate of the arcuate nucleus. In studies using

the pregnant ewe as a model, investigators could show that GHRP stimulates GRF and GH release as well as somatostatin by measuring efferent products in the effluent of portal blood of the pregnant ewe. Little is known yet about the effects of these compounds in chronic use.

Dr. C. Eschen, Copenhagen, Denmark, showed that the administration of GHRP-6, originally synthesized by Dr. Cyril Y. Bowers, to rats for 14 to 90 days had little effect on weight gain or IGF-1 levels. Several new GH secretagogues have been synthesized recently. These GHRP analogues were discussed by Professor Robinson, and were also the topic of several poster presentations at the meeting.

One of the analogues, hexarelin, was described as a potent GH releaser in children and laboratory animals such as dogs. Its usefulness in the more refined diagnosis of GH deficiency was proposed. It should be noted that only 0.3% of GHRP is absorbed via the oral route, thus limiting its potency considerably. This does not seem to be the case for newer nonpeptidal oral secretagogues such as L-692,429 and L-692,585, which have a manifold higher potency and also better absorption.

Dr. S.L. Dickson, Cambridge University, England, showed that GHRP L-692,585 was inducing *fos* protein in the arcuate nucleus. Elegant neurocytochemistry documented the induction of this key protein in the wall of the third ventricle.

The GRF neuron can best be characterized as a clearinghouse for the multiple afferent neurons. The pituitary has to be viewed as an organ with considerable plasticity. The primary function of the hypothalamus, according to Professor Robinson, is to regulate pituitary size and thus enable specific pituitary hormonal responses. In conclusion, GHRPs, which were thought to act directly on GH secretion cells in the pituitary, are now believed to produce many of their effects by interacting with somatostatin and by stimulation of GRF neurons in the hypothalamus.

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Chromosomal Localization of the Human Renal Sodium-Phosphate Transporter to Chromosome 5: Implications for X-Linked Hypophosphatemia

Phosphorus and sodium are absorbed across the luminal membrane of the renal tubule utilizing a cotransporter protein whose gene has been cloned.¹ The present investigators employed 3 methods for localizing the chromosomal site of this gene: (1) probing of somatic cell hybrid panels with a radiolabeled DNA genomic fragment that revealed a signal on human chromosome 5; (2) PCR amplification of DNA from somatic cell hybrids to localize the sodium-phosphate cotransporter gene to chromosome 5; and (3) utilizing a fluorescein-labeled DNA probe for the sodium-phosphate cotransporter gene and fluorescent in situ hybridization on metaphase chromosome spreads from human peripheral blood lymphocytes, which located the gene at chromosome 5q13. The location of the sodium-phosphate cotransporter gene on chromosome 5 was unexpected because the trait for human familial hypophosphatemic rickets in which renal phosphorus reabsorption is decreased is X-linked and has been assigned to Xp22.1-p21.3. Therefore, there may be a second phosphorus transporter isoform whose gene is on the short arm of the X chromosome, or the X chromosomal product may regulate expression of the gene on chromosome 5 or the function of its product.

United States. Although it is clearly X-linked, the gene for the function that is defective in these patients (ie, renal phosphorus transport) is not on the X chromosome. In the animal model of this disorder, the X-linked Hyp mouse, there is a decrease in the renal tubular content of mRNA and protein for the sodium-phosphate cotransporter that probably accounts for decreased renal tubular transport of phosphorus.² The chromosomal site of the mouse sodium-phosphate cotransporter gene has not as yet been reported, but there are data suggesting the presence of a humoral factor (whose gene may possibly be on the X chromosome) in these animals that inhibits phosphorus transport, perhaps by downregulating transcription of the sodium-phosphate cotransporter gene.² A "knock-out" experiment in which the calcium-phosphate transporter gene is eliminated and the effect on phosphorus transport observed would be of interest.

A second study has been published that localizes the human sodium-phosphate cotransporter gene to chromosome 5q35.³ Thus, both reports assign this gene to the long arm of chromosome 5, but its specific sublocation is not certain.

Allen W. Root, MD

Ghishan FK, et al. *Pediatr Res* 1994;35:510-513.

Editor's comment: *Familial hypophosphatemic rickets is the most common form of rickets presently encountered in the*

1. Magagnin S, et al. *Proc Nat Acad Sci USA* 1993;90:5979-5983.

2. Tenenhouse HS, et al. *J Clin Invest* 1994;93:671-676.

3. Kos CH, et al. *Genomics* 1994;19:176-177.

Turner Syndrome: Natural History, Ethnic and Genetic Influences, Methods for Evaluation of Growth

The natural history of growth in Turner syndrome (TS) has been described by several authors previously, who were mainly from North European countries. These reports have not always been consistent. In some instances, the methodology was open to criticism, so that evaluating the effects of growth hormone treatment relied on insufficient data. Four papers published in the same issue of a European journal¹⁻⁴ afford useful contributions to solve the discrepancies reported.

The first paper¹ gives data recorded from a multicentric, retrospective, nationwide study (29 pediatric endocrinology centers) of 772 cases of TS in Italy. A major purpose was to present standards and charts for birth weight and height, and weight from infancy to adulthood, appropriate for this area. The study took into account the parents' height and the birth length of TS patients. The size of the study permitted the calculation that a 10 cm difference in midparental heights between 2 groups, one shorter than the other, resulted in a 6.5 cm difference in the adult stature of a TS patient. The data also indicated that birth lengths and weights strongly correlated inversely with postnatal growth. Other data obtained from this study were that the span and frequencies of the karyotypes of the 772 cases of TS did not differ from that reported in other series. There was a slightly but significantly increased incidence of TS with the age of both parents and the mother's parity.

The second paper² reports longitudinal data from the 1st to

the 18th year of life, obtained from a subgroup of these Italian patients, with calculation of their annual growth velocity. Taking into account the different karyotypes, this study shows that most TS subjects with 46,XX mosaicism have some degree of spontaneous puberty, occurring in the same age range as in normal girls, and earlier than in other karyotypic groups of TS. There was a slight pubertal growth spurt followed by a deceleration, so that in this series the final height was a little below that of 45,X patients. Charts for height and for growth velocity have thus been established separately for TS subjects with karyotypes 45,X, 46,XX/45,XO mosaicism, and 46,XX with structural abnormalities of the second X chromosome.

The third paper³ reports the spontaneous final heights of 216 TS subjects from the southern part of France. In this series, no significant differences between karyotypic groups were found. The correlation with midparental height was $r=0.45$, a little stronger with father's height than with mother's height. Pointing out the importance of genetic factors and studying the differences recorded in TS statistics from various countries, the authors conclude that the results of treatment with growth hormone in TS must take into account the midparental height of each patient.

The last paper⁴ is a critical approach to the methods for evaluating growth in TS, especially when appreciating the effectiveness of treatment with growth hormone. Analyzing 13

different studies of growth velocity and height in TS, the authors stress their discrepancies and point out the influence of auxometric methodologies when calculating the standard deviations (SD) to the means. They show that the variations of SD applied to TS growth tables and charts affect the evaluation of therapeutic results in these patients to a large extent. Their conclusion is that, in the absence of a completely accurate method for appreciating changes in growth velocity, the investigations on the effects of GH on growth in TS should focus mainly on final heights after therapy.

1. Bernasconi S, et al. *Acta Paediatr Scand* 1994;83:292-298.
2. Mazzanti L, et al. *Acta Paediatr Scand* 1994;83:299-304.
3. Rochiccioli P, et al. *Acta Paediatr Scand* 1994;83:305-308.
4. Haeusler G, et al. *Acta Paediatr Scand* 1994;83:309-314.

Editor's comment: These 4 studies afford useful data and comments for improving the detailed knowledge of growth in Turner syndrome, its relationships with parents' height, with birth length and weight, with the spontaneous partial breast development occurring in certain Turner patients, with the karyotype, and for developing cooperative studies with the aim of a relevant evaluation of short-term therapeutic results. These publications can be considered as milestones in preparing appropriate methodologies for new longitudinal studies. The data included in the manuscripts are much more extensive and detailed than could be abstracted here. Readers who are particularly interested should make every attempt possible to read the original articles.

Jean-Claude Job, MD

Perspectives of Longitudinal Growth in Cystic Fibrosis From Birth to Adult Age

Haeusler et al performed a retrospective longitudinal analysis of growth data of 139 patients (72 girls, 67 boys) with cystic fibrosis (CF) who received care at the University of Vienna between 1955 and 1989. Height was measured with a Harpenden stadiometer and 1,605 individual observations were utilized. The mean observation period was 7.1 ± 5.9 years. The mean number of recorded height and weight measurements for each patient was 10.3 ± 7.6 . Age at diagnosis was 1.4 ± 2.37 years in girls and 1.5 ± 2.56 years in boys. In all cases a demonstration of *Pseudomonas aeruginosa* was treated vigorously with antibiotics. Dietary management included 150% of daily allowance with high dose supplementation of pancreatic enzymes. Fat was not restricted. Quartiles of height, weight, and growth velocity were estimated by nonparametric measures.

Height and weight were available in 103 patients. At birth, weight and length of children with CF were decreased compared to healthy infants. As expected, there was a further decrease in weight SDS in both girls and boys between birth and the time of diagnosis. Length, moderately decreased at birth (-0.55 ± 0.13 SDS in girls; -0.39 ± 0.13 SDS in boys), declined until diagnosis. During the year after diagnosis, length SDS improved but remained decreased. In girls, height followed the 25th percentile until age 8, when it dropped to the 10th percentile until approximately age 14. At that time, it began to rise to the 25th to 50th percentile. Median height of 18 girls who were available for measurement at 19 years was between the 25th and 50th percentile. Weight in girls showed a similar course. In boys, height followed the 25th percentile with a nadir between 10 and 16 years. The median height of 13 boys available at 19 years was 173 cm (25th percentile). Weight was much more affected than height in girls than boys after age 12. Growth velocity was relatively normal during the prepubertal age. Puberty was delayed. The calculation of growth velocity during puberty was not possible due to too few data. However, the pubertal growth spurt appeared to be both small and delayed.

There was no significant change in height SDS or growth velocity in either boys or girls after the acquisition of latent or established respiratory infections.

Haeusler G, et al. *Eur J Pediatr* 1994;153:158-163.

Editor's comment: This is an interesting report particularly as it covers a time span of 34 years between 1955 and 1989. One might suspect that treatment of children with CF was vastly different nearly 30 years ago, and that height and growth velocity of these children would be markedly different from that of the normal population. In addition, one might anticipate that the acquisition of *P. aeruginosa* infection would significantly reduce growth velocity. Neither of these suppositions proved correct. It is noteworthy, however, that for this relatively large group of children, mean height was reduced from the 50th percentile. One can only speculate that current aggressive therapy might have produced improved growth in these individuals.

William L. Clarke, MD

In Future Issues

Osteochondrodysplasias With Mild Clinical Manifestations:

A Clinician's Guide

by Richard M. Pauli, MD

Noonan Syndrome: A Review

by Michael A. Patton, MA, MSc, MD

Prader-Willi Syndrome: The Unfolding Genetic Story

by Uta Francke, MD

Imaging In Diagnosing Hypopituitarism by Raphael Rappaport, MD

Rationale for Recommending that Recombinant Human Growth Hormone be Prescribed by Weight Rather Than Units

by Margaret H. MacGillivray, MD

Calcium-Sensing Receptor Genes Mutate and Produce Metabolic Disease

Ca^{2+} associated with a specific cell membrane component had been postulated for several years to explain, in part, the mechanism by which Ca^{2+} regulates the secretion of parathyroid hormone,¹ but the structure of the membrane component was unknown until Brown and coworkers isolated the bovine gene for this receptor from parathyroid tissue and expressed and characterized it. The bovine gene encodes a 120 kd, 1,085 amino acid, 7 transmembrane polypeptide characteristic of receptors that activate guanyl triphosphate (GTP) binding proteins. This activation initiates a cascade of intracellular signals that produce the characteristic biologic response of the cell to the ligand. The receptor is expressed in the bovine parathyroid gland, kidney, thyroid, and some areas of the brain.

Subsequently, the 6 exon of the human gene was isolated and mapped to chromosome 3q2. It encodes a 1,059 amino acid with an extremely long (613 amino acids) amino terminal extracellular region to which Ca^{2+} is thought to bind. Hypothesizing that the Ca^{2+} receptor gene was abnormal in patients with familial hypercalcemic hypocalciuria, the composition of this gene was analyzed in patients with this disorder and its more severe variant, neonatal severe hyperparathyroidism. All of the affected members of the families studied had base pair changes, although the genetic error varied in different families. Three variants were identified. In the amino terminal region, a G → A mutation in codon 186 altered arginine to glutamine and a C → T mutation in codon 298 changed wild-type glutamine to lysine. These mutations might affect Ca^{2+} binding to the receptor or alter polypeptide processing, receptor stability, or other necessary function. In the third intracellular domain, a C → T mutation in codon 796 altered arginine to tryptophan; this amino acid is near the site of receptor coupling to the GTP-binding protein. One subject with severe, and often fatal,

neonatal hyperparathyroidism had 2 copies of the abnormal gene at codon 298, thus lacking 2 functional Ca^{2+} receptor molecules. These observations suggest that familial hypercalcemic hypocalciuria is due to abnormalities within the gene coding for the membrane Ca^{2+} receptor and is genetically heterogeneous.

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2. Pollak MR, et al. *Cell* 1993;75:1297-1303.

Editor's comment: These articles provide an important advance in our understanding of the manner in which Ca^{2+} regulates the secretion of parathyroid hormone. Acting through the membrane Ca^{2+} receptor, Ca^{2+} activates GTP-binding proteins that increase activity of phospholipase C, thus hydrolyzing membrane phosphoinositide and increasing intracellular concentrations of inositol triphosphate, thereby releasing Ca^{2+} from its storage sites in calciosomes; Ca^{2+} and the GTP-binding proteins may also "open" Ca^{2+} channels directly. As the intracellular concentration of Ca^{2+} increases, neutral proteases, termed "calpains," are activated and increase the rate of degradation of parathyroid hormone.² High intracellular Ca^{2+} levels also decrease the rate of transcription of the gene for parathyroid hormone. It is possible that an abnormality in the Ca^{2+} receptor may account for the parathyroid hyperplasia seen in patients with multiple endocrine neoplasia or in a clone of parathyroid cells leading to a parathyroid adenoma. These unique observations suggest that there may be membrane receptors for other ions as well (ie, K^+ , MG^{++}).

Allen W. Root, MD

Genetic Mapping of Quantitative Trait Loci for Growth Fatness in Pigs

Quantitative inheritance of a trait implies that the expression of that trait is dependent on the interaction of several genes at different loci and, often, environmental factors. In order to identify the chromosome(s) on which the traits for growth and fatness in pigs may reside, the investigators analyzed the quantitative trait loci (QTL) in second generation crossbred progeny of European domesticated pigs (selected for large growth and leanness) and the European wild boar (characterized by increased body fat content but smaller size) utilizing a linkage map and genetic markers for the porcine genome of 18 autosomes. The authors measured birth weight, growth rate, abdominal and back fat, and length of the small intestine (a trait that correlates positively with growth) and reported that wild boar alleles on chromosome 4 were associated with decreased growth, shorter small intestinal length, and increased body fat content. There was also a QTL for birth weight and early growth on chromosome 13. There was no relationship between the detected QTLs and sex or feeding. The precision of chromosomal location of these QTL is relatively low; therefore, the authors could not determine whether these chromosomal sites contained 1 or multiple genes affecting the quantitative trait.

The loci for the genes for growth hormone (chromosome 12), its receptor (chromosome 16), and insulin-like growth factor 1 (chromosome 5) were not related to the QTL for growth, body fat, or intestinal length. Loci corresponding to porcine chromosome 4 are found on chromosome 1 in humans.

Andersson L, et al. *Science* 1994;263:1771-1774.

Editor's comment: The relevance of these observations to obesity in humans is uncertain, but it is of interest to note that in the mouse there are 2 genetic mutations associated with an obese phenotype (diabetes on mouse chromosome 4 and fat on chromosome 8) linked to genes with homologues on the first human chromosome. This observation points to a potential focus of attention in the study of human obesity and growth. The lack of association of body fat content with feeding regimen points to the importance of genetic factors in fat accumulation. It was a bit surprising that growth was not genetically linked to the loci for growth hormone, its receptor, or IGF-1, particularly since growth hormone-deficient pigs are dwarfed.

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GROWTH

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Noonan Syndrome: A Review

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Now a well-established entity, Noonan syndrome (NS) is a frequent cause of short stature. The syndrome is estimated to have an incidence of 1 in 2,500 to 1 in 1,000 individuals.¹ This incidence, which is not based on complete ascertainment in a defined population, may appear high; however, NS is being increasingly recognized and is likely to be the second commonest syndrome with congenital heart disease after Down syndrome. Unlike Down syndrome, NS has a familial basis.

The syndrome was clearly defined by Dr. Jacqueline Noonan in 1963.² In 1968, she subsequently described both male and female patients with a characteristic facies, pulmonary valvular stenosis, and short stature.³ There had previously been confusion about the status of male patients with the apparent features of Turner syndrome, but it was then recognized that NS accounted for cases described as male Turner syndrome. Although the term NS is most frequently used, some authors still use the term Ullrich-Turner phenotype synonymously.

CLINICAL FEATURES (Table 1)

The most significant clinical abnormality in most children is the presence of congenital heart disease, which is found in up to 80% of patients. The most frequent heart defect is pulmonary valvular stenosis, which is usually isolated but may be associated with other structural abnormalities. The electrocardiogram shows a superior axis, which reflects an abnormality in the conducting system. Frequently there is a structural deformity of the chest wall, with pectus carinatum superiorly and pectus excavatum inferiorly.

Of considerable interest is the presence of hypertrophic cardiomyopathy in up to 20% of patients.⁴ Histologic examination of cardiac muscle has shown

Table 1
Clinical Features of Noonan Syndrome

Pulmonary valvular stenosis	62%
Hypertrophic cardiomyopathy	20%
Abnormal electrocardiogram	87%
Height (<10th centile)	70%
Undescended testes	77%
Feeding difficulties (severe)	24%
Developmental delay (mild)	10%
Refractive errors	67%
Coagulation abnormalities	60%
Ptoxis (severe)	42%
Pterygium colli (webbed neck)	23%
Pectus carinatum/excavatum	95%

a pattern of myofibrillar disarray similar to that seen in other forms of inherited cardiomyopathy.⁵ This would suggest that children with NS would be at increased risk from sudden cardiac arrhythmias, but a recent report by Burch et al⁶ has not confirmed this. They demonstrated the presence of hypertrophic cardiomyopathy in the neonatal period, which may lead to cardiac failure and has up to a 20% mortality in this period. The overall natural history of cardiomyopathy in NS is unclear, and certainly a

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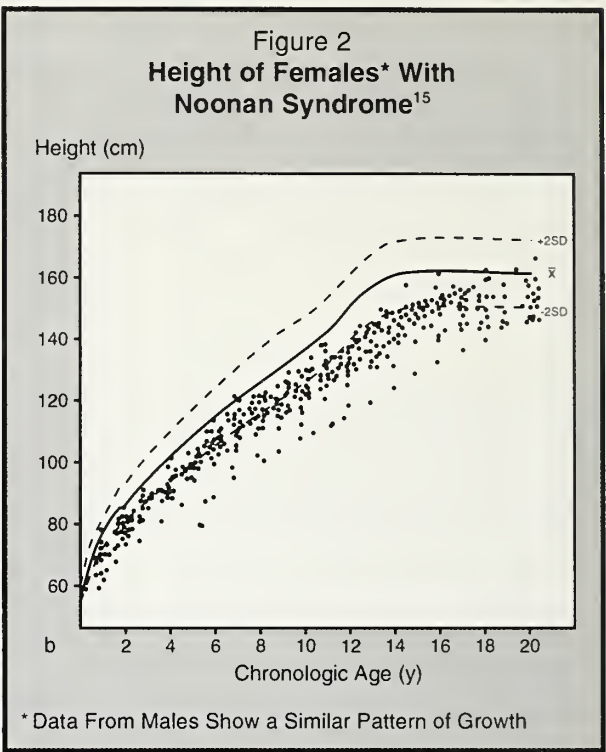
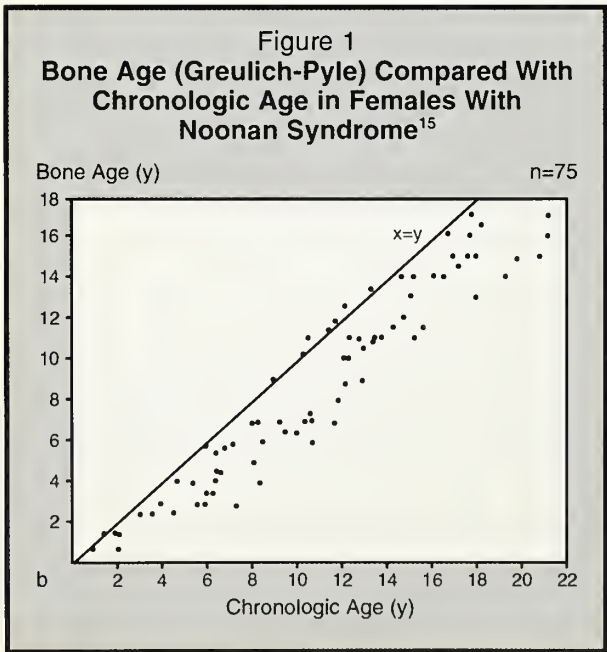
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few cases appear to improve or even resolve during childhood.

The characteristic facial features include hypertelorism; down-slanting palpebral fissures; epicanthal folds; ptosis; low set, anteriorly rotated ears; and neck webbing. In addition, coarse curly hair and keratosis pilaris (excessive keratinization of the hair follicles) may occur.⁷ The facial features change with age⁸ and tend to become less marked in later childhood and adult life.⁹

One of the unusual and poorly understood features of this syndrome is the association with abnormal bleeding. Severe, life-threatening bleeding is fortunately rare, but a history of troublesome post-operative bleeding or easy bruising is found in 65% of patients.¹⁰ The nature of the bleeding disorder differs among affected individuals. Witt et al¹¹ have shown a combination of clotting factor and platelet deficiencies in 19 patients with NS. De Haan et al¹² demonstrated partial factor XI deficiencies in 9 patients. In a more extensive study of 72 individuals with NS, Sharland et al¹⁰ demonstrated defects in the intrinsic coagulation factors. They showed 33% had factor XI.C deficiency, 17% had factor VIII.C deficiency, and 14% had factor XII.C deficiency. In some cases there were combined deficiencies of 2 or more factors. These coagulation factor deficiencies were independent of the cardiac abnormality and were not associated with liver failure or excessive consumption of clotting factors. These intriguing observations have yet to be explained, and it remains to be seen how the gene mutation in NS can modify the levels of coagulation factors.

Feeding difficulties are frequent in infancy. Severe feeding difficulties (defined as tube feeding for 2 weeks or longer in a term infant) were present in



24% of cases.⁴ These difficulties are independent of the cardiac abnormality and resolve during early childhood. Refractive errors and strabismus are also frequent and need to be identified early and corrected.¹³

GROWTH AND DEVELOPMENT

The birth weight in NS falls within the normal range, although some babies may be generally edematous at birth. After birth, the growth in height and weight usually lie below the 3rd centile, while the head circumference continues to lie within the normal range. This gives the appearance of relative macrocephaly. The mean (standard deviation [SD]) delay in bone age, assessed by the Greulich and Pyle method, is -2 years (Figure 1).⁴ Several studies on the growth of children with NS have been carried out, and growth charts are available in the papers by Witt et al¹⁴ and Ranke et al¹⁵ (Figure 2). Growth velocity as compared with Turner syndrome also is presented in the paper by Ranke et al¹⁵ (Figure 3).

Growth hormone (GH) has been used to correct short stature in NS, but it has often been used in individual patients without standardized protocols. It may be important to monitor ventricular wall thickness in such cases, since there is some anecdotal evidence that cardiomyopathy may increase while on treatment. One study using GH 28 IU/m²/wk, in 14 children with NS has shown an increase in growth velocity from 3.8 to 10.5 cm/y after 1 year of treatment. Ventricular wall thickness was monitored in this study before and during treatment using

standardized echocardiographic sections, and no pathologic increase in ventricular wall thickness has been noted.¹⁶ A further study of 5 children treated with GH for a period from 1.8 to 4.6 years has also shown a favorable response.¹⁷ Puberty is delayed in both males and females with NS. The mean \pm SD age of menarche in 20 women with NS was 14.6 ± 1.17 years. In males, the onset of puberty is more difficult to define, but it is frequently considerably delayed.¹⁸ In addition, undescended testes are present in 60% to 80% of affected males. Fertility appears to be normal in females, but up to 50% of males are infertile. In one study of 8 adult males with NS, infertility was not associated with normally descended testes or a unilateral undescended testis but was associated with bilateral undescended testes, even following surgical correction.¹⁹

A number of earlier reports on NS suggested an association with mental deficits; however, recent series have shown this is rarely the case. Motor milestones are often delayed (eg, sitting unsupported, mean = 10 months; walking unsupported, mean = 21 months), but only approximately 10% of patients require special schooling for learning difficulties. The presence of mental retardation in NS indicates the need for careful chromosome studies, since a number of chromosomal abnormalities produce a similar phenotype.

GENETICS AND ETIOLOGY

Clearly, NS is often an autosomal dominant disorder, since vertical transmission from father to son has frequently been demonstrated. There is considerable variation in expression, and on occasion it

may be milder in its expression in the parent or grandparent than in the child. This makes genetic counseling difficult if there are no striking features of NS in either parent. In the majority of such cases, the condition will have arisen as a new mutation; however, since there is a recurrence risk of 5% in those cases in which neither parent shows signs of NS, it is possible that some individuals have the mutation without any significant physical stigmata.

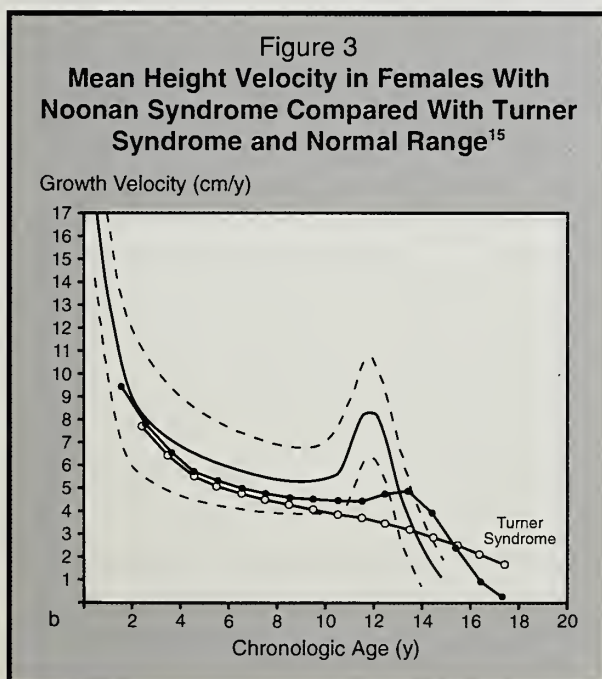
The gene locus for NS has not yet been mapped, and the gene product is not identified. The gene may exert part of its phenotypic effect through pre-natal hydrops, since this has been described in a number of case reports and also has been suggested in the pathogenesis of neck webbing in Turner syndrome. However, it is difficult to see how this mechanism alone could account for the diverse phenotypic features.

There are a number of syndromes to consider in the diagnosis of NS. These include cardiofaciocutaneous syndrome, leopard syndrome, Watson syndrome, and neurofibromatosis (NF)/NS (neurofibromatosis plus). In the Watson syndrome, patients have pulmonary valvular stenosis, café au lait patches, and reduced intelligence. Both Watson syndrome and NF/NS have been shown to be due to mutations (often with large deletions or rearrangements) in the NF1 gene on 17q11.^{20,21} Interestingly, the NF1 locus was excluded early in the linkage studies on NS.²² It may be that different loci are modifying similar embryologic pathways.

In conclusion, the diagnosis of NS still rests on its clinical features. A diagnostic scoring system²³ is available to assist in the diagnosis, but it is hoped that molecular genetic studies will ultimately help in confirming the diagnosis and elucidating the heterogeneity, if any, of the syndrome.

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Prader-Willi Syndrome: Chromosomal and Gene Aberrations

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Prader-Willi syndrome (PWS) was first recognized as a clinical entity in 1956 by Andrea Prader, a pediatric endocrinologist in Zurich who, together with his colleagues Labhart and Willi, described 9 patients with obesity, short stature, cryptorchidism, and oligophrenia following severe hypotonia in the newborn period.¹ The etiology of the condition was unknown. Since the vast majority of cases are sporadic, the recurrence risk is estimated to be low (1.6%), making a mendelian pattern of inheritance unlikely. It was hypothesized that a primary developmental defect in the hypothalamus could be responsible for the clinical findings (Table 1). When chromosome studies were performed in these patients, the karyotypes were interpreted as normal in most cases; however, starting in 1976 various abnormalities involving chromosome 15 were reported. They were of different types, including robertsonian and reciprocal translocations, both balanced and unbalanced; isochromosomes for the long arm; and the presence of additional small metacentric markers derived from chromosome 15. Because of the rare occurrence and inconsistent nature of these karyotypic abnormalities, no straightforward hypothesis of PWS's being due to a chromosomal imbalance could be derived from these observations. Finally, in 1981 Ledbetter and colleagues,^{2,3} using high-resolution chromosome analysis, reported finding small interstitial deletions of the 15q11-q13 region in a high proportion of these patients. Submicroscopic deletions or mosaicism were speculated to be present in those PWS cases whose karyotypes were apparently normal. However, structural abnormalities involving this region are difficult to interpret, particularly using very high-resolution chromosome analysis. There is often a difference in appearance between the maternal and paternal chromosomes due to differential condensation of the proximal 15q region. Therefore, under high-resolution analysis, chromosomes from normal individuals may show differences between the 2 chromosome 15 homologues in the number and size of subbands in this region. Molecular cytogenetic diagnosis, in particular in situ hybridization with probes from the commonly deleted region, provides more definitive evidence for deletions than cytogenetic analysis of elongated chromosomes.

The etiologic relationship between the apparent chromosome 15 deletion and the PWS phenotype was challenged when, in 1987, an identical deletion was reported in a number of patients with a neurologic disorder quite different from PWS, called Angelman syndrome (AS) after the author of the first report in 1965. The features include microcephaly; jerky movements; seizures; a peculiar face with prominent chin; large mouth with protruding tongue; and inappropriate laughter. Mental deficiency in AS patients is usually more severe than in PWS and

Table 1
Criteria for Clinical Diagnosis of Prader-Willi Syndrome¹⁹

Major Criteria

Neonatal/infantile central hypotonia
Feeding problems/failure to thrive in infancy
Rapid weight gain after 12 months
Facial features: narrow bifrontal diameter, almond-shaped eyes
Hypogonadism
Mild/moderate developmental delay
Hyperphagia

Minor Criteria

Decreased fetal movement; infantile lethargy, improving with age
Behavior problems; obsessive/compulsive, rigid, stubborn
Sleep disturbance/apnea
Short stature by age 15 years
Hypopigmentation
Small hands and feet for height age
Narrow hands with straight ulnar border
Esotropia, myopia
Thick viscous saliva
Speech articulation defects
Skin picking

Supportive Findings

High pain threshold
Decreased vomiting
Temperature control problems
Scoliosis and/or kyphosis
Early adrenarche
Osteoporosis
Unusual skill with jigsaw puzzles
Normal neuromuscular studies

the dysmorphic, neurologic, and behavioral findings are quite distinct. There are more familial cases in AS, and the recurrence risk in siblings was estimated as 4%. The deletions of the 15q11-q13 region in PWS and AS appear cytogenetically similar if not identical.⁴

Resolution of this puzzle was provided in 1983 when it was discovered that deletions in PWS always involve the paternally derived chromosome 15⁵ while deletions in AS always involve the maternally derived chromosome.⁶ Furthermore, in a significant proportion of PWS patients whose chromosomes appear structurally normal, both copies of chromosome 15 are maternally derived; the paternal copy is missing (uniparental disomy, UPD).⁷ In AS, a smaller fraction of cases have paternal UPD and about one third of AS cases have neither deletion nor UPD. Submicroscopic deletions that are maternally inherited have been demonstrated in some families with multiple affected sibs. In other families with inherited AS, no deletion has been demonstrated. It is likely, therefore, that only 1 or very few genes are responsible for the AS phenotype, that they are expressed exclusively from the maternal chromosome, and that they are silent on the paternally derived chromosome.

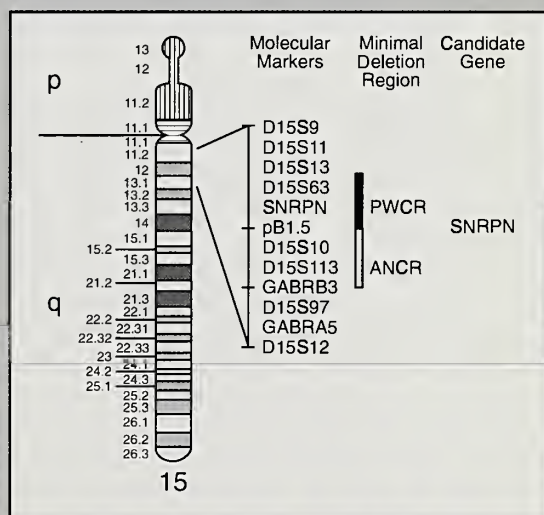
The situation for PWS is quite different, as there are very few familial cases and all sporadic cases have either UPD or deletions. It is likely, therefore, that PWS is a true microdeletion syndrome that requires the silencing or deletion of more than 1 locus from the paternally derived chromosome 15. Nevertheless, the extent of the deletions in the majority of PWS and AS cases is quite similar on the molecular level. The breakpoints are defined in intervals between molecular markers and the total region is approximately 3 to 5 mega-base pairs in size (Figure 1). This entire region, however, behaves differently on the maternal versus the paternal chromosome in normal individuals. For example, evidence is emerging that the region replicates earlier on the paternal than on the maternal chromosome⁸ and that there are differential DNA methylation patterns.⁹ Taken together with the different clinical features of the 2 deletion syndromes and the observation of UPD, the differential replication and methylation strongly suggest that there are genes in this region that are predominantly or exclusively expressed from only 1 chromosome 15, ie, they carry a parental-specific imprint.

Are the genes responsible for PWS and AS intermingled, or are there distinct subregions that may be responsible for the different phenotypes? The discovery of a few unusual patients who appear to have the full syndrome in the presence of submicroscopic partial deletions has allowed researchers to subdivide the region into a PWS minimal deletion region (PWCR) and a distally adjacent AS minimal

region (ANCR) (Figure 1). The submicroscopic deletion reported in a 3-generation Japanese family¹⁰ is particularly informative because a woman who inherited this deletion from her father was phenotypically normal; in particular, she did not have any PWS features. In 3 of her children, who inherited the chromosome 15 with the deletion, the features of AS were present. The deletion in this family delineates the ANCR. The deletion junction fragment flanking the deletion (pB1.5) demarcates 1 border of the PWCR.¹¹ The proximal border is defined by another unusual patient with a partial deletion. The entire PWCR has been cloned in overlapping yeast artificial chromosomes. It is 320 kb in size.¹²

The first gene encoding a known protein mapped to the PWCR was SNRPN, the gene for small nuclear ribonucleoprotein (snRNP)-associated polypeptide SmN.¹³ Expression of SNRPN appears to be limited to neuronal tissue.¹⁴ The protein sequence is highly similar to SNRPB (gene on chromosome 20), which is ubiquitously expressed. In the brain, however, SNRPB expression is replaced by SNRPN. By studying DNA from PWS patients with partial deletions, SNRPN was mapped to the minimal PWS deletion region (Figure 1).¹³ The

Figure 1



Ideogram of chromosome 15²⁶ with ordered molecular markers within the common Prader-Willi syndrome/Angelman syndrome (PWS/AS) deletion region (15q11.2-q13.1). The minimal deletion region for PWS (PWCR) extends from a breakpoint between D15S63 (PW71) to pB1.5¹¹ and for AS (ANCR) from pB1.5 to a breakpoint within GABRB3, the gene for the β_3 -GABA receptor.

homologous locus in mouse, *Snrpn*, was mapped to a region on mouse chromosome 7 that is evolutionarily related to the human 15q11-q13 region. In mouse brain, the *Snrpn* gene was expressed exclusively from the paternal allele, as one would expect for a gene involved in the PWS phenotype.¹⁵ To determine whether the gene is also imprinted in humans, extensive sequencing of the human SNRPN gene was carried out in normal human samples and has revealed a common sequence polymorphism in exon 2, where either a C or T is present.¹⁶ This polymorphism allows the determination of the parental origin of any transcripts. In brains from fetuses who were heterozygous at the DNA level, it could be shown that only the paternal allele is expressed.¹⁷

By virtue of its location in the PWCR and its uniparental expression from the paternal chromosome, SNRPN is the premier candidate gene to explain the PWS phenotype. The cDNA is rather small, consisting of 720 nucleotides that encode 240 amino acids and are distributed over 7 coding exons. The total genomic size is less than 25 kb. To prove that SNRPN is a candidate for PWS, a mutation in the paternally derived copy of the gene needs to be demonstrated. However, PWS in the absence of either deletion or UPD is extremely rare. In 2 such rare families,¹⁸ sequencing of the SNRPN coding regions from affected individuals has not yet turned up a mutation. Likewise, patients with some features of PWS but who do not meet the diagnostic criteria (Table 1)¹⁹ have been studied for SNRPN deletions or gene rearrangements, with negative results.¹³ Inactivation of SNRPN, however, could be due to mutations outside of the coding region.

SNRPN is also an attractive candidate gene based on functional considerations. It is a core protein of snRNP particles that also contain small nuclear RNA molecules and are involved in the processing of pre-mRNA or the transport of mRNA out of the nucleus. The highest expression of SNRPN is in the brain, which suggests that N-containing particles may have a brain-specific function, eg, brain-specific splicing of differentially spliced genes such as calcitonin. Speculations about the pathogenetic mechanism of SNRPN deficiency assume that in the absence of SNRPN expression, no N-containing snRNPs would be made. The absence of these particles might not affect all of the central nervous system neurons equally but may be particularly important for areas of the hypothalamus, where centers responsible for the regulation of muscle tone, growth, appetite control, temperature control, and pain sensitivity — all systems affected in PWS — are located.^{13,15}

Diagnostic tests for PWS have progressed from high-resolution cytogenetics, which is tedious and

the results of which are often difficult to interpret, to the use of molecular technology. In particular, fluorescence in situ hybridization (FISH) is now routinely used in diagnostic cytogenetic laboratories. In this procedure, large insert probes that hybridize consistently to normal chromosome 15 and fail to hybridize to PWS chromosomes in which the hybridizing region is deleted are used. While probes from the larger commonly deleted region (Figure 1) can be used successfully in the majority of deletion cases, it is preferable to use probes containing the SNRPN gene to ensure detection of rare cases with partial deletions of the region. FISH results have to be correlated with the phenotype, since a deletion that is detected cannot be assigned to the maternal or paternal chromosome by this method. In the absence of a deletion, molecular genetic studies such as polymerase chain reaction (PCR) analysis of highly polymorphic dinucleotide repeats are necessary to determine the presence of UPD.²⁰ This requires parental samples. UPD can be detected by distinct restriction fragments generated by methylation-sensitive enzymes and hybridization with probes from the region.^{9,21} Another novel direct test currently being developed makes use of blood samples to study the expression of SNRPN and another paternally expressed gene in the PWS region (IPW). By PCR analysis of reverse transcribed mRNA, expression is detected in normal controls but not in PWS patients with either deletion or UPD.²²

As is true for much of the recent progress in human molecular genetics, the identification of a gene involved in a particular genetic disorder is only the first step towards understanding the pathogenesis of the disease manifestations. Such an understanding is necessary in order to devise rational treatment protocols that would interfere with the stepwise process that leads from the abnormal gene to the diseased brain. In the case of PWS, a promising candidate gene for the hypothalamic manifestations, SNRPN, has been identified. Most PWS patients, however, have deletions that extend much beyond the PWCR and include many more genes. The hypopigmentation frequently observed²³ may somehow be related to hemizyosity at the (nonimprinted) p locus (D15S10, Figure 1), since individuals homozygous for mutations or deletions of this gene have tyrosinase-positive oculocutaneous albinism.²⁴ It is also possible that genes outside of the deleted region whose pattern of methylation, replication, and expression has been altered by the deletion contribute to the PWS phenotype.^{25,26} It may be necessary, therefore, to identify all genes in the region that are potentially involved before one can propose hypotheses for pathogenesis and design experiments to address them.

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Rationale for Dosing Recombinant Human Growth Hormone by Weight Rather Than Units

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The practice in Europe and Japan of prescribing growth hormone (GH) on the basis of units differs from that in the United States and Canada, where milligram dosing has been the standard procedure since 1985. The consequence of these 2 approaches to dosing human GH (hGH) is widespread confusion in the literature as well as in the medical community. Many clinicians are not familiar with the events that led to this dual approach to prescribing GH. This article will discuss the factors that influenced how hGH was initially prescribed and the new technologies that have greatly improved its purification and characterization. Fortunately, we now have a much clearer understanding about the relationship between unitage and weight for recombinant hGH (rhGH). Hence, dosing by milligrams per kilogram of body weight should become the universal norm for prescribing and writing about

rhGH. Failure to move in this direction will mean that it will be impossible to compare studies done in North America with those done elsewhere. Even in North America there was recent confusion caused by claims that one rhGH product was more potent than another on the basis of unitage comparisons; the erroneous basis for this conclusion will also be explained in this article.

In this report, the historical basis for dosing rhGH by units is reviewed along with the developments that have led to the current practice in North America of using milligrams.

HISTORICAL ASPECTS

Although pituitary GH was first identified in 1921, it was not until 1956 that a GH preparation became available for clinical use in hypopituitary patients.¹⁻⁵ Much early effort had been dedicated to isolating bovine GH and porcine GH and to documenting their anabolic and metabolic properties in hypophysectomized rats. When these GH products were administered to humans, they were ineffective; the treatment failures were presumed to result from catabolic impurities or toxic contaminants.^{6,7} However, studies in comparative endocrinology provided insight into species specificity of GH. Specifically, fish GH prepared by Wilhelmi was ineffective in rats but anabolic in fish. Bovine GH and porcine GH were anabolic in rats but ineffective in monkeys, while monkey GH led to nitrogen retention in hypophysectomized monkeys.⁸⁻¹⁰ Successful collections of human pituitary glands by Li et al in San Francisco and Raben in Boston resulted in the availability of human pituitary GH (phGH), which proved to be efficacious in human hypopituitary patients.^{5,11}

In 1963, the National Pituitary Agency was established to improve the collection, extraction, and distribution of hGH.¹² The method used by Wilhelmi and colleagues to extract native phGH from

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by Raphael Rappaport, MD

acetone-preserved or frozen pituitaries differed from the ones used by Raben (glacial acetic acid) and Li $[(\text{NH}_4)_2\text{SO}_4]$.^{2,3,13,14} Those early preparations of native phGH contained variable amounts of impurities and degraded products. Additional purification by column chromatography was not undertaken until 1977 because losses from this additional step would have aggravated the existing severe shortages of hormone.¹⁵ It was essential in this period to standardize phGH using bioassays because of product variability; moreover, sensitive techniques for characterizing the physicochemical properties of hormones had not been developed. Hence, dosing of phGH by arbitrarily defined units of activity derived from bioassay standardization was the only available choice at that time.

From 1956 to 1985, the main bioassays used to standardize phGH were the hypophysectomized rat weight-gain assay and the tibial width assay.¹⁶⁻¹⁸ In both bioassays, 2 doses of international GH standard (10 μg and 50 μg) and 2 doses of "unknown" GH product were administered subcutaneously to hypophysectomized rats, and the estimated relative potency of the unknown was calculated versus the international standard using the mean gains in each group of test animals over the entire treatment period. In the weight-gain assay, the gain in body weight is proportional to the logarithm of the daily dose of GH over a range of 10 to 250 $\mu\text{g}/\text{d}$. In the tibial width assay, the increased width of the proximal epiphysis of the tibia is proportional to the log of the total dose of GH administered in micrograms over a range of 15 to 400 μg . Although other bioassays were developed during this period, the only one that is current and considered the *gold standard* is the rat weight-gain assay.

Then in 1985, the Food and Drug Administration (FDA) approved the first rhGH preparation (somatrem*) for clinical use and mandated that dosing be based on the physicochemical property of weight (therefore, milligrams), provided the product had been standardized for biologic activity. The bioassay method employed was the weight-gain assay in young hypophysectomized rats. The potency of rhGH was estimated by comparing its effect to that of an international reference standard (bovine pituitary GH for bioassay, World Health Organization [WHO]). Using this approach, somatrem was assigned a biopotency of 2 IU/mg and the dose approved for clinical use was 0.3 mg/kg/wk.

Confusion about reported differences in the potency of the 2 available rhGH products, somatrem* (methionyl hGH) and somatropin† can be traced to the use of different primary GH standards for standardization of their biologic activity. Historically, bovine pituitary GH standard was used until 1987 to

standardize phGH as well as somatrem. The desirability of an international standard of like origin led to the development of a primary human pituitary standard 80/505 for bioassay in 1987 (potency 2.6 IU/mg). Unlike somatrem, somatropin, which was the second rhGH to be approved, was standardized using the phGH standard 80/505. It was assigned a specific activity of 2.6 IU/mg, while somatrem, which had been initially standardized using the original bovine GH standard, had received a specific activity of 2.0 IU/mg. Restandardization of somatrem using the phGH standard 80/505 proved that somatrem and somatropin are equipotent, ie, 2.6 IU/mg.

CURRENT CONSIDERATIONS

Why is it still necessary to standardize rhGH for biologic activity? The FDA reasons that rhGH will be produced by different processes, which may or may not alter the biologic activity of the product, and has mandated that bioassay standardization will remain one of the prerequisites for drug approval.¹⁹ Recently, the argument has been made that while bioassay standardization was essential for natural biologic preparations because they gave highly variable responses in animals or cultured cells, recombinant DNA-derived products have a much higher level of purity (>99%) and can be dosed accurately by weight alone. The FDA insists, however, that potency (activity determination) is still needed because physicochemical methods in themselves are unable at present to guarantee the biologic activity of a protein derived from natural or recombinant sources. It is likely that a further adjustment in the potency of rhGH products will occur because of the development of a new international WHO rhGH reference reagent for somatropin (88/624) by the National Institute for Biological Standards and Control (NIBSC).²⁰ The proposed specific activity of this rhGH reference reagent will be approximately 3.0 IU/mg. However, due to considerable disagreement within the scientific community about the validity of this specific activity, the new preparation will not be designated as a primary reference material but will be assigned a potency of 6.7 IU/ampule and 2.0 mg/ampule and serve as a reference reagent. Based on this issue, a separate United States Pharmacopeia (USP) standard for rhGH is presently being developed. It is also possible that more accurate biochemical methods will become available for determining potency and that the concept of units of activity based on relatively imprecise bioassays will eventually be eliminated. For example, sophisticated in vitro receptor binding assays or specific antibody epitope mapping methods may eventually prove to be acceptable alternatives to the current animal bioassays.

In January 1993, a panel of experts participated in a GH workshop convened by the FDA for the purpose

*Protropin

†Humatrope

of selecting the most suitable tests for evaluating and labeling rhGH products.¹⁹ The panel was asked to choose which tests should be used to determine the identity (proof of authentic hGH structure); the purity (lack of contaminants and degraded products); and the chemical strength (quantity of authentic hGH in milligrams of rhGH). A majority of the panel recommended reversed phase high-performance liquid chromatography (RP-HPLC) and peptide mapping as identity tests, although some panel members supported the use of SDS-PAGE and size exclusion chromatography (SEC) as alternative or additional methods of determining identity of hGH. There was unanimous disapproval of amino acid analysis as an identity test. With regard to purity testing, the panel agreed that RP-HPLC, SEC, ion exchange, and SDS-PAGE are satisfactory methods. The tests recommended for evaluating the chemical strength were ultraviolet spectrophotometry, SEC, and occasionally RP-HPLC in the event of ultraviolet interference. Many of the methods now available for characterizing the physicochemical properties of rhGH will ensure a level of hormone purity not possible in the phGH era.

CONCLUSION

Many countries (eg, European countries and Japan) continue to require rhGH dosing in units while vial contents are labeled in milligrams. Regardless of how rhGH is dosed elsewhere, the FDA will adhere to its policy that rhGH products be dosed in milligrams provided bioassay criteria are satisfied. Given the worldwide availability of precise and sensitive methods for ensuring production of equipotent rhGH products, a uniform approach to dosing by milligrams would eliminate the confusion that exists

when comparisons are made of treatment regimens in different countries.

ACKNOWLEDGMENTS

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Abstracts From the Literature

Utah Growth Study: Growth Standards and the Prevalence of Growth Hormone Deficiency

This population study aimed to: (1) determine the feasibility of having nonmedical personnel accurately perform and record height measurements; (2) obtain serial height measurements on randomly selected school-age children to establish norms by age and sex for height and growth rates; (3) generate growth curves for comparison with standard growth curves; and (4) determine the prevalence of growth hormone deficiency (GHD) as a cause of growth failure in the study population.

In order to achieve these goals, a prospective investigation was designed in the state of Utah. In 251 elementary schools, randomly selected by computer, 114,881 children were measured the first year; 79,495 growth rates were calculated after the second measurements obtained 1 year later. The height and growth velocity curves generated were very similar to the currently used charts (National Center for Health Statistics growth charts). Subsequently, 555 children with short stature (<3rd percentile) and poor growth rates (<5 cm/y) were examined. The presence

of GHD (defined as peak level <10 ng/dL with 2 provocative tests) was found in 16 children who were previously undiagnosed; 17 children were already known to have GHD. The male:female ratio was 2.7: 1 ($P=0.006$). Six girls with Turner syndrome were identified.

Among the authors' conclusions were that the growth curves generated in the 1960s and 1970s are valid for children of the 1990s and that most children growing <5 cm/y (a commonly used threshold rate) will not have an endocrine disorder. If the criterion were shifted to <4.5 cm/y, none of the children with GHD would have been missed and fewer children with normal variants of short stature would have been examined. In many children (48% in this study), GHD and Turner syndrome may currently be unrecognized and untreated. GHD appears to be more common in boys. The incidence of GHD in Utah and, presumably, in the United States is at least 1/3,480.

Lindsay R, et al. *J Pediatr* 1994;125:29-35.

Editor's comment: This very good and much needed study is based on a large population and offers an estimated incidence of GHD among elementary school children in Utah. It also confirms the validity of some of the currently used norms. However, there are several methodologic problems that limit the completeness of ascertainment, summarized by the authors as follows: (1) Volunteers, though carefully trained, may have committed undetected errors. (2) Growth rates were obtained for only 69% of the original base of 114,881 children. Therefore, some slow-growing children from the original group may have been missed. (3) Some children with growth problems were never examined because of lack of parental concern or refusal to allow follow-up. (4) The diagnostic evaluations made by the physicians were not uniform. (5) Children of normal stature and low growth rate were not examined.

Although these self-criticisms are well evaluated, probably the most important deficiency of the study is not mentioned. The weight and weight progression of the children were not measured and followed up. Weight and height are 2 parameters that need to be considered together for the evaluation of growth. Linear growth will not take place in the absence of appropriate weight gain. Since weight was not considered in this study, nutritional growth retardation was not diagnosed, nor was it considered by the authors.

In this study, primary care physicians seemed to miss 85% of

the children with growth failure. However, just as important is the failure of endocrinologists to look for and diagnose nutritional growth retardation, a condition that may be more prevalent than GHD.

Fima Lifshitz, MD

Second Editor's comment: Indeed, this is an important article. In my opinion, the most important aspect of this article is that it demonstrates the importance and value of screening programs to identify pathology that is not otherwise recognized. Objections to screening programs are running rampant, and even highly respected endocrinologists vary in their opinions. Concerns have been expressed about unnecessarily creating anxiety among parents of normal but short children. Granted that anxiety may be unnecessarily created; however, the positives of identifying many children with unrecognized organic causes of growth retardation outweigh the negatives, in my opinion. It is particularly important to identify children who are <3 standard deviations below the mean in height, as 50% of these will have an organic cause for short stature, and to identify those growing less than 4.5 cm/y, as pointed out by Lindsay et al.

Robert M. Blizzard, MD

Developmental Timing of Dynamic Mutations

Dynamic mutations differ from classic mutations in that the DNA change occurs once and is usually passed on exactly the same. Dynamic mutation is the term used to describe the change (increase or decrease) of DNA sequences resulting from the amplification of naturally occurring polymorphic trinucleotide repeats (Sutherland et al, 1992). In other words, this is a new and different type of mutation, and it is not stable. The mutation begins as small increases in the copy number, but the number is outside of the normal range. The increases may vary; therefore, they may be different sizes in different cells, and these may increase or decrease in size from one generation to the next. Some of the disorders associated with dynamic mutations are fragile X syndrome, myotonic dystrophy (MD), and Huntington disease (HD).

An excess number of repeats (CGG) in the Xq23.7 region has been reported in the fragile X syndrome. The normal number of repeats is 6 to 54. The premutations range from 52 to more than 200, and the mutation contains more than 200 repeats (Mandel, 1993). In fragile X syndrome, the person who carries the least number of trinucleotide repeats but who does not have the typical phenotype is said to be the carrier of a premutation. When passed from generation to generation, this premutation is unstable and may expand over a few generations, gradually increasing in size when transmitted by females but remaining the same when transmitted by males. When transmitted by a female and increased in size, it may become a full-blown mutation, which is clinically significant since it produces the typical fragile X syndrome phenotype with mental retardation.

HD is an adult-onset, progressively neurodegenerative disorder that presents with choreic movements, psychiatric changes, and intellectual deterioration. The mode of inheritance is autosomal dominant. Recent findings have shown that

the genetic defect in HD is related to an excess number of tandem GAC repeats in the chromosome 4p16.3 region due to allelic expansion. The number of tandem repeats in normal individuals is between 11 and 24. In contrast, individuals with HD have 42 to 86. The most elongated repeats are associated with individuals who acquired HD through a new mutation. It has also been shown that the length of repeats correlates with onset and severity of disease, and if the gene is paternally inherited, the phenotype appears earlier and is more severe.

In some disorders the number of copies is directly related to the age of onset of the disease. The congenital form of MD for example, is related to the largest number of repeats (Harley et al, 1992). The same situation occurs in HD, in which the juvenile-onset form is also associated with the largest number of repeats (Andrew et al, 1993). In Kennedy disease, the age of onset is inversely related to the increase in copy number. Another neurodegenerative disorder associated with triplet repeats is hereditary dentatorubral-pallidoluysian atrophy (DRPLA) (Miwa, 1995).

A recent article by Sutherland et al (1993) reviews the latest findings regarding dynamic mutations. The authors suggest that their occurrence may be able to explain such concepts as incomplete penetrance, variable expression, and anticipation. The fact that all of the disorders described so far have been associated with triplet repeats and are neurodegenerative has led to the suggestion of a common mechanism for neuronal degeneration caused by unstable expansion of these repeats. The functions of the genes, however, remain unclear.

The mutation seen in fragile X syndrome has been shown (Wohrle et al, 1993) to vary in repeat length in different tissues (somatic mosaicism). The constancy of the repeat in HD was not known. In order to determine the degree of mosaicism and

the stability of the repeat, MacDonald et al (1994) studied 4 pairs of monozygous twins affected with HD and the sperm from HD gene carriers. The 4 pairs of monozygous twins had identical repeat lengths. Different repeat lengths (germline mosaicism) were detected in the sperm of HD carriers. When the repeat lengths of the HD carriers' sperm was compared with the repeat lengths in their lymphoblasts, it was found that the sperm DNA had longer repeat lengths and that the greatest degree of gametic mosaicism was found with the longest somatic repeats.

Because of the somatic variation seen in the repeats in fragile X syndrome it had been suggested that repeat expansion may occur during early embryogenesis. MacDonald et al concluded that even though allelic expansion may be a common underlying mechanism for HD and fragile X syndrome, the developmental timing of the instability of the repeats is different.

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Editor's comment: Using the term mutation for these disorders is not completely accurate. In the strictest sense of the word these mutations are not really mutations, but new and different

mechanisms that result in disease. Another important finding of the study of the association of trinucleotide repeats and neurodegenerative disorders has changed the methods of finding genes. As in the case of DRPLA, the gene for other neurodegenerative disorders that have been mapped to a specific chromosome may be found by looking for trinucleotide repeats.

Judith G. Hall, MD

Second Editor's comment: Evidence has been increasing that the presence of large blocks of trinucleotide repeats disrupts the transcription of genes in which they reside; however, the mechanisms involved have not been clear. The articles cited here provide insight not only into how this might happen but also why disorders associated with such blocks exhibit a threshold effect with regard to appearance of the clinical phenotypes. Indeed, as Wang et al point out, the presence of very strong nucleosome positioning signals where they do not normally reside may not only interfere with the expression of relevant genes but may also disrupt copying of the trinucleotide repeats by DNA polymerase, which could lead to further expansion of the repeats, another feature of these disorders. The article by Wang and colleagues also reminds us that we must keep our minds open and to look for new mechanisms to explain how mutations cause genetic disease.

William A. Horton, MD

Sucralfate Causes Malabsorption of L-Thyroxine

Following their experience with a hypothyroid woman in whom the dose requirement for L-thyroxine increased substantially after sucralfate, a nonabsorbed aluminum salt of sucrose sulfate prescribed for treatment of dyspepsia, Sherman and colleagues studied the effect of this agent on absorption of L-thyroxine. In healthy adult volunteers, 80% of an ingested dose of 1,000 µg of L-thyroxine was absorbed. When L-thyroxine and sucralfate were administered simultaneously, only 23% of ingested L-thyroxine was absorbed, and the rate of absorption slowed. When L-thyroxine was administered 8 hours after a dose of sucralfate, 78% of administered L-thyroxine was absorbed. Thus, the authors concluded that sucralfate impairs the absorption of L-thyroxine, probably by intraluminal binding of the hormone.

Sherman SI, et al. *Am J Med* 1994;96:531-535.

Editor's comment: Several medications inhibit intestinal absorption of L-thyroxine, including ferrous sulfate, aluminum hydroxide, and colestipol, as well as sucralfate, an agent that is utilized for duodenal ulcers, gastritis, reflux esophagitis, and dyspepsia. Sucralfate also impairs absorption of tetracycline, phenytoin, and digoxin. Although in children and adolescents poor compliance with medication intake is the most common cause of erratic dose requirements for L-thyroxine and other medications, it is important to remember when confronted with this problem that one drug may adversely affect the absorption, serum protein binding, excretion, or pharmacokinetics of another agent.

Allen W. Root, MD

Snaring the Achondroplasia and Hypochondroplasia Gene

Achondroplasia (ACH) is the most common human chondrodysplasia. A milder form of disproportionate short stature that also has rhizomelic shortening and normal bone histology is hypochondroplasia (HCH).

The estimated incidence of these disorders is 1 in every 15,000 live births, and until now, their etiology has been unknown. Individuals affected with ACH have short (rhizomelic) limbs; macrocephaly; depressed nasal bridge; lordosis; and short, stubby trident hands. HCH presents with short stature but few clinical symptoms, and the radiologic findings are similar but milder than

those seen in ACH. Ninety percent of ACH and HCH cases are the first affected individual in the family. In the case of ACH, an association has been made with increased paternal age, as is often seen in de novo mutations. Affected individuals are fertile, and the disorder is passed on as an autosomal dominant trait.

In early 1994, the gene for ACH was localized to the tip of the short arm of chromosome 4 (4p16.3). Ironically, the locus mapped very near to another elusive disease gene locus, the Huntington disease (HD) gene locus. Three independent groups reported the localization.

Velinov et al studied 14 families with 40 DNA markers distributed randomly throughout the genome. When 1 of these showed a positive lod score, indicating possible linkage near the end of the short arm of chromosome 4 (4p), 10 other DNA markers were tested that mapped to the same region on chromosome 4. One marker showed a maximum lod score of 3.65. A lod score of more than 3.0 is generally accepted as demonstrating genetic linkage between a disorder and the chromosomal location identified by the marker. Multipoint linkage analysis, in which linkage of several markers is tested simultaneously, suggested that the gene most likely resided within a 4-cM genetic region distal to the HD locus at chromosome 4p16.3.

Le Merrer and colleagues investigated 6 families with ACH and 9 families with HCH. Because of the similar features, HCH has been suspected of being an allelic form of ACH. A lod score of 3.01 was established for the ACH families alone, and a lod score of 4.71 for the ACH plus HCH families combined. Importantly, when a computer program called HOMOG was applied to test the hypothesis that the 2 conditions are genetically homogeneous, the results suggested that they were, ie, they are allelic disorders. Francomano et al studied 18 families with ACH using 6 DNA markers that map to chromosome 4p. This analysis yielded maximum lod scores of 6.44 and 9.9, using 2-point and multipoint linkage analysis, respectively.

The 3 papers firmly placed the ACH locus within a region about 2.5 Mb of DNA at the tip of the short arm of chromosome 4. All 3 papers also pointed out that among known genes that map to this area, a fibroblast growth factor receptor gene (FGFR3) was a good candidate. Fibroblast growth factors (FGFs) transmit growth signals to cells in many tissues, and FGFR3 was known to be expressed in cartilage. However, despite this insight, most workers in the field did not expect the ACH locus to be identified quickly, perhaps biased by the many years it had taken to find the HD locus in the same region. Thus, it came as a surprise when Shiang et al reported 2 mutations in FGFR3 in several patients with ACH.

The authors (Shiang et al) used denaturing gradient gel electrophoresis (DGGE) to screen FGFR3 cDNA fragments derived from lymphoblasts and fibroblasts. When DGGE results from homozygous ACH patients and controls were compared, a sequence difference was suggested in a fragment corresponding to the transmembrane domain of the molecule. The investigators next sequenced this region in the ACH DNA, and found a consistent G to A base change at nucleotide 1138 of the FGFR3 coding sequence. This base change results in an arginine substitution for a glycine at position 380 of the mature protein.

The authors recognized that this base change also creates a cleavage site for the restriction enzyme *SfcI* that is not present in the sequence of the normal allele. This finding enabled them to easily distinguish the 2 alleles on the basis of the presence or absence of the restriction site. With this strategy, they analyzed genomic DNA from their patient material, which included 6 unrelated cases of homozygous ACH, the heterozygous ACH parents of 2 of these homozygotes, and 2 sporadic heterozygous ACH cases — for a total of 16 ACH chromosomes — and more than 50 controls.

The results showed that 5 of the 6 homozygous ACH patients were homozygous for the 1138 G to A base change and that all of the sporadic cases were heterozygous for this base change. It was not detected in any of the controls. Thus, 15 of 16 ACH chromosomes exhibited the same base, which was not found in controls. Further analysis of DNA from the homozygous ACH

infant in whom only a single copy of the 1138 G to A mutation was present revealed that the same 1138 G was changed to a C instead of an A. This change also results in an arginine substitution for glycine at position 380 in the mature gene product.

Thus, the same base, 1138 G, was changed in 16 of 16 ACH alleles examined and causes the same amino acid substitution in the transmembrane domain of FGFR3 in all cases.

The authors addressed several issues in their discussion. First, they pointed out that the G at nucleotide position 1138 must be extremely mutable. Second, they suggested that the glycine to arginine change at amino acid residue 380 of the mature receptor protein may be specific for the ACH phenotype. Third, they speculated on mechanisms by which such mutations might operate, including interference with FGF signal transduction through FGFR3 receptors and possibly through other FGF receptors. Finally, they stressed that the high frequency of this mutation should facilitate prenatal diagnosis in couples at risk for homozygous ACH.

Francomano CA, et al. *Hum Mol Genet* 1994;3(5):787-792.

Le Merrer M, et al. *Nat Genet* 1994;6:318-321.

Shiang R, et al. *Cell* 1994;78:335-342.

Velinov M, et al. *Nat Genet* 1994;6:314-317.

Editor's comment: After many years of pursuit and at least 1 false start, the search for the ACH gene appears to be over. Indeed, although yet to be published, the 1138 G to A mutation that causes a glycine to arginine substitution in the transmembrane domain of FGFR3 has been confirmed by others in an extremely high proportion of patients with typical ACH. This saga is of interest from many perspectives. For example, the relative merits of positional cloning versus candidate gene analysis strategies are often debated vigorously among gene hunters. The extremely rapid unfolding of this story demonstrates how the 2 strategies can be effectively coupled. It also underscores the value of characterizing the human genome to the extent possible so that when a given disease is linked to a particular chromosomal region, candidate genes can be readily identified and analyzed.

From a biologic perspective, the results raise as many questions as they answer. First, the reasons why the 1138 G should be so mutable are far from clear. The authors point out that the mutation occurs in the context of a CpG dinucleotide. C to T transitions are known to occur frequently in this setting, especially if the C is methylated, since the latter can become deaminated to a T, changing a G:C base pair to an A:T base pair. Even taking this phenomenon into account, the mutation rate for this nucleotide is extremely high. One wonders if factors not evident at this time are involved. It seems likely that further characterization of the human FGFR3 gene will provide insight into this question.

Similarly, despite a rapidly growing knowledge of FGF ligands, receptors, signaling pathways, and the relevance of FGF signaling to limb development, it is not at all evident how substituting an arginine for a glycine in the transmembrane domain of FGFR3 disrupts bone growth or how this specific change produces such a specific phenotype. For example, FGFR3 is expressed in many tissues, especially the brain. Why should abnormalities of FGFR3 signaling be restricted to skeletal development? Likewise, it is probably reasonable to assume that the FGF signaling defect responsible for ACH resides in the growth plate. However, could the defective signaling involve vascular cells that invade the growth plate or other cells, such

as perichondrial or bone marrow cells, that somehow influence growth plate function? What is clear is that much more work is needed to sort out the molecular pathogenesis of ACH.

Finally, it is somewhat comforting to discover that ACH mutations map to a growth factor receptor gene, since it has long been suspected that the basic defect in this condition involves growth plate regulation.

William A. Horton, MD

Second Editor's comment: The fact that deletions of the distal arm of chromosome 4 have been reported in Wolf-Hirschhorn syndrome and that the clinical findings are very different to those seen in ACH and HCH suggests that ACH and HCH may not occur due to dosage effect, but rather to the negative effect of the mutant gene. These findings suggest the possibility of prenatal diagnosis.

Judith G. Hall, MD

Phenotype Specific RET Oncogene Mutations and Multiple Endocrine Neoplasia Syndromes

Multiple endocrine neoplasia (MEN) syndromes constitute a family of disorders characterized by neoplasias of 2 or more endocrine tissues. Medullary thyroid carcinoma (MTC) is a tumor of the thyroid C cells that can occur sporadically or as part of the inherited cancer syndromes MEN IIA, MEN IIB, and familial MTC (FMTC).

Individuals with MEN IIA are predisposed to C-cell hyperplasia or MTC, pheochromocytoma, and hyperparathyroidism. Individuals with MEN IIB are predisposed to mucosal neuromas and marfanoid habitus.

The loci for MEN IIA, MEN IIB, and FMTC have been mapped to an interval on chromosome 10q11.2. The RET proto-oncogene is also located in this region. The RET proto-oncogene is a receptor tyrosine kinase gene expressed in MTC and pheochromocytoma and in normal thyroid and adrenal tissue.

Mulligan et al reviewed 118 unrelated families with inherited MTC for mutations of the RET proto-oncogene. They found mutations in 1 of the 5 cysteines of the proto-oncogene in 97% of patients with MEN IIA and in 86% of the patients with FMTC, but not in the MEN IIB patients. Eighty-four percent of the MEN IIA mutations affected codon 634, and patients with a Cys634 to

Arg substitution had a greater risk of developing parathyroid tumors than those with other codon 634 mutations.

They concluded that the precise location of the mutation corresponds with the clinical phenotype and that mutations in the 634 codon may be predictive in families predisposed to adrenal or parathyroid disease. The basis of the tissue specificity of these RET mutations is unclear, but the authors suggested the possibility of tissue-specific differences in RET expression or in RET protein interactions.

Mulligan LM, et al. *Nat Genet* 1994;6:70-75.

Editor's comment: It is not absolutely clear that there is just 1 mutation for MEN. The identification of a mutation for phenotype-specific RET mutations is important for early screening in individuals known to be at risk. Prenatal screening for the RET mutation, however, may prove to be controversial and cause serious ethical and moral dilemmas, since the decision to terminate a pregnancy is always difficult. This is especially true in adult-onset diseases.

Judith G. Hall, MD

A Single Amino Acid Substitution in the Exoplasmic Domain of the Human Growth Hormone (GH) Receptor Confers Familial GH Resistance (Laron Syndrome) With Positive GH-Binding Activity by Abolishing Receptor Homodimerization

The absence of detectable growth hormone-binding protein (GHBP) was believed for some time to be a constant feature of the Laron syndrome. However, Aguirre et al (*Acad Sci Paris* 1990;311:315-319) and Buchanan et al (*Clin Endocrinol* 1991; 35:179-185) described patients with classic Laron dwarfism except for the presence of high-affinity serum GHBP activity. Duquesnoy et al report in this article the following: (1) In 2 unrelated families, the same GH receptor (GHR) mutation was identified as the mutation resulting in the substitution of a highly conserved aspartate residue by histidine at position 152 of the exoplasmic domain. (2) The genetic analysis was consistent with a founder effect, ie, common origin, for this mutation. (3) The GHR mutant retains GH-binding capability and is correctly expressed at the plasma membrane. (4) The mutant GHR has lost reactivity of 1 mAb epitope, which is supposed to belong to the region where both receptor molecules contact each other, suggesting that the D152H substitution interferes

with the dimerization process and GHR activity. They conclude that these in vitro data, along with the phenotype observed in vivo in the proband patients, provide further support for the 3-dimensional model of the exoplasmic domain of GHR that has been produced in *Escherichia coli*.

Duquesnoy P, et al. *EMBO J* 1994;13:1386-1395.

Editor's comment: Geneticists and genetically oriented pediatricians will find this article to be of much technical and clinical interest. The authors apply multiple refined techniques to demonstrate and elucidate the conclusions reported. The original article is lengthy by necessity, but worth reading page by page for many scientific and clinical reasons. This journal is available in many university libraries.

Robert M. Blizzard, MD

Growth After Renal Transplantation in Prepubertal Children: Impact of Various Treatment Modalities

The authors retrospectively evaluated growth in 47 prepubertal boys and 23 prepubertal girls following renal transplantation. The data were analyzed with respect to several variables, including initial growth retardation, type of immunosuppressive therapy (azathioprine versus cyclosporine), alternate-day versus daily prednisone, and total prednisone dose. Data were collected from the start of the first dialysis for up to 2 years after the first renal transplant. Height, weight, sexual maturation, serum creatinine, episodes and type of dialysis, and the number of renal transplants were also recorded. The primary renal disease of these children included glomerulopathies (50%), urinary tract abnormalities and/or renal hypoplasia (36%), and nephrotic syndrome (11%).

The mean height for all subjects was below the 3rd percentile at the start of the first dialysis and decreased significantly during the dialysis period. At the time of the first renal transplant, the mean height standard deviation score (SDS) for boys was -3.0 and -2.3 for girls. Following transplantation, height SDS increased by +0.3 in boys but decreased by -0.1 in girls. Catch-up growth did not occur over the next 2 years in 70% of these children. Gender, duration of initial dialysis, age at first renal transplantation, and the duration of a glomerular filtration rate (GFR) of <50 mL/min/1.73 m² were not associated with a change in SDS. But a significant positive association between height SDS and the percentage of time on alternate-day prednisone therapy was noted. Likewise, a positive association between the extent of urinary tract abnormalities and/or renal hypoplasia versus other types of renal disease was demonstrated. By using backward multiple regression analysis, the authors showed that percentage of time on alternate-day prednisone therapy, cumulative dose of prednisone, azathioprine versus cyclosporine, and duration of reduced GFR had a significant negative influence on height SDS 2 years after transplantation.

Hokken-Koelega A, et al. *Pediatr Res* 1994;35:367-371.

Editor's comment: Accurate knowledge of the possibility of spontaneous catch-up growth after kidney transplantation in children with chronic renal glomerular insufficiency is extremely important for interpreting the results of therapeutic trials with growth hormone (GH) and establishing appropriate indications. The homogeneity of the strictly prepubertal cohort followed for 2 years, the relevance of the auxologic data, and the quality of the statistical analysis make this paper valuable. One may regret that the endocrine data regarding GH secretion, insulin-like growth factor 1, and plasma binding proteins in these patients, if available, were not included in this work.

Jean-Claude Job, MD

Second Editor's comment: The authors summarize their data in some detail. They note that height SDS was already significantly decreased at the time dialysis was initiated and few children (30%) had catch-up growth after renal transplantation. Certainly these data suggest that alternative treatments are needed to stimulate growth in children with chronic renal disease post-transplantation. At the present time, biosynthetic GH has been approved for the treatment of growth retardation in children with chronic renal insufficiency, and trials are underway to demonstrate its effectiveness post-transplantation. Van Dop et al (*J Pediatr* 1992;120:244-250) have shown marked acceleration of growth rates (from 1.9 to 7.2 cm/y) in a group of post-transplant children treated with GH with a mean bone age of 8.9 ± 2.7 years. Hopefully, studies such as these will change the outlook for children with renal transplants with regard to stature.

The readers may wish to read the next abstract dealing with growth post-transplant in adolescents treated with GH.

William L. Clarke, MD

Growth Hormone Treatment in Growth-Retarded Adolescents After Renal Transplant

Administration of recombinant human growth hormone (rhGH) (10.8 or 21.6 mg/m²/wk in divided daily doses) to slowly growing, short adolescents with renal failure who had been recipients of a renal transplant for more than 1 year increased growth rate approximately 4-fold during the first year of treatment. During 2 years of therapy, the height increment of 15.7 cm realized by the rhGH-treated patients was 10 cm greater than that achieved by historical control subjects matched for chronologic age, sex, pubertal stage, time after renal transplantation, and immunosuppressive regimen. Bone age increased 0.8 years for each year of therapy with rhGH. The linear growth response was not related to the dose of rhGH administered, the pretreatment growth rate, or alternate-day as compared with daily prednisone administration (0.10 to 0.25 mg/kg/d), but was greater in subjects who were in early rather than late stages of adolescence at the initiation of therapy. Administration of rhGH had no adverse effect on glomerular filtration rate or effective renal plasma flow on the entire group beyond that experienced by the control subjects. However, there was an increased incidence of deteriorating renal function in patients receiving

alternate-day (6/11) rather than daily (1/7) prednisone therapy with rhGH, the reason for which was not clear. Serum concentrations of insulin-like growth factor (IGF)-1 and IGF-binding protein (IGFBP)-3 increased. IGFBP-1 did not change, and IGF-2 declined during administration of rhGH. The investigators conclude that rhGH increases growth rates in adolescent recipients of renal transplants and may increase final height without untoward effects on graft function, particularly in patients receiving daily prednisone therapy.

Hokken-Koelega ACS, et al. *Lancet* 1994;343:1313-1317.

Editor's comment: GH has long-term stimulatory effects on the growth of children with chronic renal insufficiency and has received regulatory approval for use in these patients.¹ The present report indicates that it may also be useful in adolescents after renal transplantation. Hokken-Koelega et al (see previous abstract) also studied the growth of 70 prepubertal children with chronic renal failure during the first 2 years after renal transplantation and reported that in 70% the growth rate

did not accelerate after surgery. Factors that impacted adversely on growth after renal transplantation were daily prednisone therapy, a high cumulative dose of prednisone, use of azathioprine rather than cyclosporine as an immunosuppressive, and a glomerular filtration rate $<50 \text{ mL/min/1.73 m}^2$. However, as noted above, alternate-day prednisone administration together with rhGH was associated with an increased incidence

of declining renal function, implying that a daily dose of prednisone may be preferable when the 2 agents are administered concurrently.

Allen W. Root, MD

¹Fine RN, et al. *J Pediatr Endocrinol* 1994;7:1-12.

Diabetes Insipidus With Impaired Osmotic Regulation in Septo-optic Dysplasia and Agenesis of the Corpus Callosum

The authors reviewed the histories and presentations of 24 children with septo-optic dysplasia (SOD) and/or agenesis of the corpus callosum; 8 had the complete form of the syndrome (optic nerve hypoplasia, abnormal septum pellucidum, and pituitary deficiency); 21 had the incomplete form, having only 2 of the triad; and 3 had isolated agenesis of the corpus callosum with pituitary deficiency. Seven were completely blind; 8 had partial but significant visual impairment; although not stated in the article, the remaining 9 were presumably able to see without significant visual impairment. Thirteen were moderately or severely developmentally delayed. Twenty had growth hormone deficiency; 15 were documented to have corticotropin deficiency; and 10 of 14 were demonstrated to be luteinizing hormone/follicle-stimulating hormone deficient when tested with gonadotropin hormone-releasing hormone.

Particular attention in the article was given to the 9 patients with diabetes insipidus (DI). These 9 had a high incidence of mental retardation (7), blindness (7), and 1 or more episodes of hypoglycemia in infancy (4). Three presented with severe hyponatremia in the first 2 months of life, and a fourth before 1 year of age. Five manifested an impaired sense of thirst in the presence of hyponatremia. Treatment, even with antidiuretic hormone, was difficult in these 5. Five had 1 or more admissions

with seizures and impairment of consciousness associated with mild hyponatremia while on antidiuretic hormone therapy.

There was little correlation between the clinical features and the structural central nervous system lesions. Seventy-eight percent of the patients with DI had incomplete forms of SOD.

Masera M, et al. *Arch Dis Child* 1994;70:51-53.

Editor's comment: The authors make a contribution to our knowledge of SOD by reporting 21 patients seen with the entity over 21 years at the Institute of Child Health in London. The incidence of seeing 1 new patient per year in a large center reflects the fact that this entity is not common, but it is certainly one we all have been or will be exposed to. The data presented here permit us to make better prognoses and to provide better care for patients with the syndrome. This is a treacherous disease — particularly in those with DI and/or blindness. It may be worth mentioning that normal, delayed, and precocious sexual development occur in SOD patients in approximately equal numbers. (Hanna CE, et al. *AJDC* 1989;143:186-189).

Robert M. Blizzard, MD

Changes in Body Composition of Children With Chronic Renal Failure During Growth Hormone Treatment

The success of growth hormone (GH) in increasing growth velocity (GV) in patients with chronic renal failure (CRF) has been reported previously. The present study was designed to investigate the changes in body composition during the first year of GH treatment in 8 CRF patients, 4.5 to 12.5 years of age, who had normal GH release to pharmacologic stimuli. The dose of GH was 0.125 IU/kg/d , which is the approximate equivalent of 0.30 to 0.35 mg/kg/wk .

Although the mean (\pm standard deviation [SD]) height increased from $108.3 \pm 12.2 \text{ cm}$ to $114.6 \pm 12.7 \text{ cm}$, the mean GV increased from $4.0 \pm 0.7 \text{ cm/y}$ to $6.3 \pm 1.1 \text{ cm/y}$, and the mean weight increased from $20.1 \pm 5.8 \text{ kg}$ to $22.9 \pm 5.9 \text{ kg}$, data pertaining to bone mineral density (measured by dual photon absorptiometry), fat body mass, and lean body mass did not change. An unexplained but intriguing finding was a distinct fall in total body potassium.

Vaisman N, et al. *Pediatr Nephrol* 1994;8:201-204.

Editor's comment: More studies regarding the effect of GH on the body composition of patients with CRF are needed. Food and Drug Administration approval has been given for its use on

the basis of increasing GV. We now need to learn about its effect on body composition. These authors have made a start in this respect.

Robert M. Blizzard, MD

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Idiopathic Prepubertal Short Stature Is Associated With Low Body Mass Index

Body mass index (BMI) was considered in 79 prepubertal children, 46 boys and 33 girls aged 3 to 12 years, followed for short stature below -1.4 standard deviations (SD). According to the results of BMI calculation compared with normal BMI values, the children were put into 2 groups: 1 below the mean for age ($n = 53$, mean BMI = -0.9 SD) and 1 above ($n = 26$, mean BMI = +0.6 SD). Age and target height of the 2 groups were not significantly different. Height and annual growth velocity were significantly less in the low BMI group, and bone age was more delayed. Growth hormone response to stimulation tests was normal in all the patients, and not significantly different between the 2 groups. In contrast, insulin-like growth factor 1 (IGF-1), measured in the plasma without extraction by a nonequilibrium technique, was very significantly lower in the low BMI group than in the high BMI group. Significant positive correlations were found between BMI and height, growth velocity, and plasma IGF-1.

The authors concluded that children with idiopathic short stature are leaner than the normal population, and that an inadequate or insufficient nutritional intake might be a contributing factor.

Thibault H, et al. *Horm Res* 1993;40:136-140.

Editor's comment: *Much work had been done in the past to look for relationships between nutrition and growth, and many studies had found various quantitative and/or qualitative nutritional deficiencies associated with individual cases of height insufficiency. What is of interest in this study are the large number of children included; the use of BMI rather than less sensitive clinical indexes of nutrition; the positive correlation of BMI with annual growth velocity; and the contrast between the similar growth hormone response to stimulation in all children and the lower levels of plasma IGF-1 documented in the lean*

children compared with the others. It is now well established that IGF-1 levels relate more to nutritional than to hormonal conditions. However, it is regrettable that certain important points, such as the psychosocial situation and the level of physical activity, have not been mentioned as having been investigated.

The practical consequence should be to carefully consider feeding habits and simple but relevant clinical and laboratory indexes of nutrition such as BMI and IGF-1 in so-called constitutionally short children. But this does not mean that any kind of nutritional supplementation would necessarily improve the growth of such children.

Jean-Claude Job, MD

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